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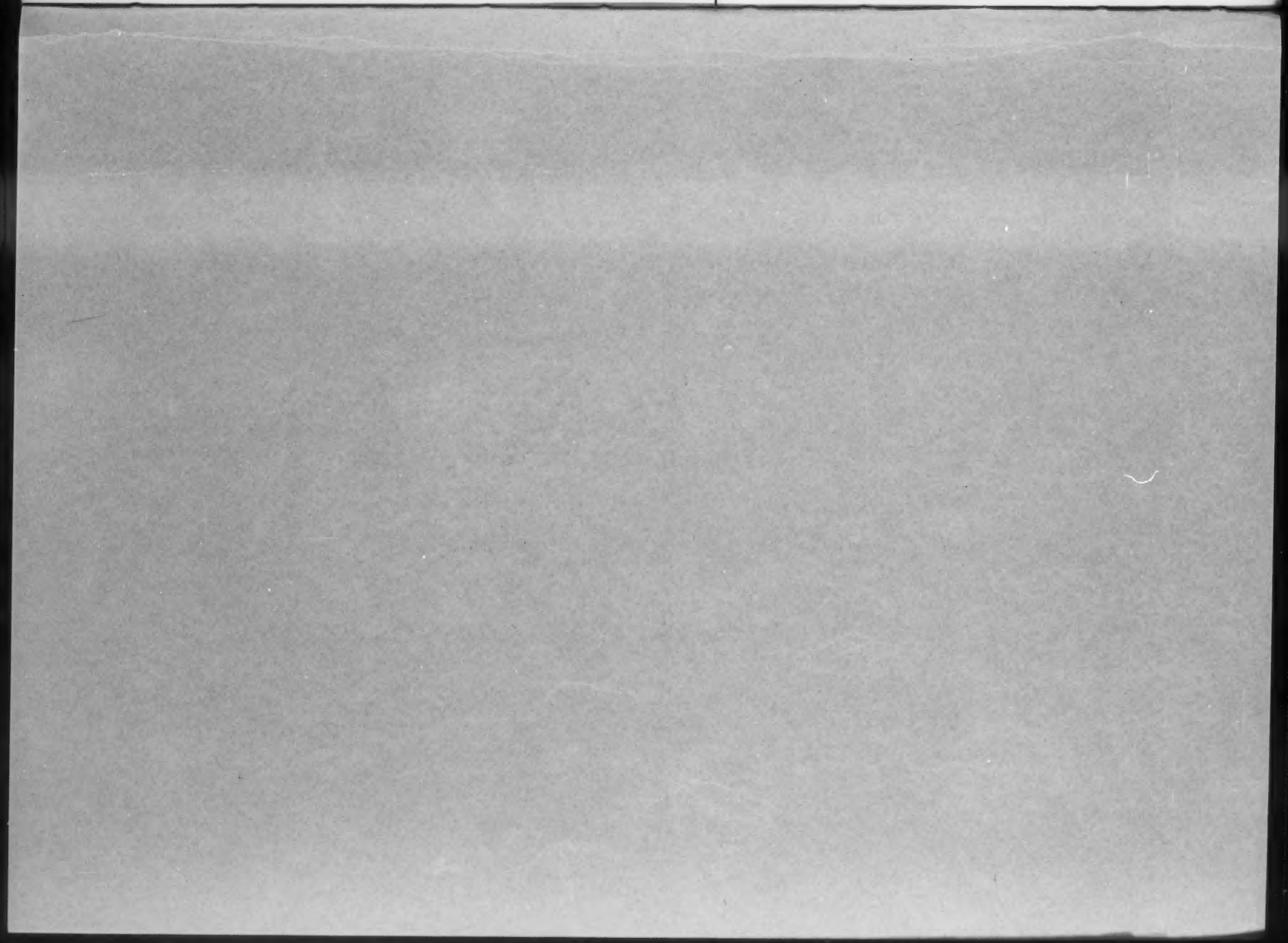
BULLETIN ON

NARCOTICS

Vol. X, No. 3, JULY-SEPTEMBER 1958

UNITED NATIONS



Bulletin on NARCOTICS

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UNITED NATIONS

EUROPEAN OFFICE

Geneva

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The consumption of narcotic drugs in Denmark

By Dr. M. Nimb

The following article has been communicated by the Government of Denmark.

This analysis is based on domestic wholesale statistics and draws a comparison between prescriptions dispensed in November 1949 and those dispensed in November 1955.

In Denmark, prescriptions for drugs subject to group I control under the international narcotics conventions cannot be repeated; that is, when the medicine has once been dispensed, the prescription is cancelled by the pharmacist and cannot be used again. The prescription for such drugs has to be entered by the pharmacist on a special form which has to be forwarded to the National Health Service at regular intervals.

The high rate of consumption in Denmark of drugs under international control has several times attracted the attention of the international control organs, which have instituted inquiries directed to the National Health Service in Denmark.

The Danish authorities, conscious of the complex nature of this problem, realized that the domestic wholesale statistics did not give adequate information on the consumption and distribution of narcotic drugs, and therefore commissioned Dr. P. Wiingaard to analyse 54,000 prescriptions for narcotics dispensed by Danish pharmacies durings November 1949.

TABLE 1

Number of prescriptions

* = *municipality*

	Nov. 1949	*Nov. 1955*	*Increase (%)*
Frederiksberg	1 803	2 272	+ 26.0
Copenhagen *	11 950	14 863	+ 24.4
Copenhagen-Frederiksberg *	4 611	7 472	+ 62.0
Frederiksborg district	1 502	2 835	+ 88.7
Holbak district	1 084	1 822	+ 68.0
Praesto district	1 433	2 296	+ 60.2
Sorø district	1 428	1 611	+ 12.1
Bornholms district	378	593	+ 56.8
Maribo district	1 610	1 978	+ 22.9
Odense district	2 963	4 284	+ 44.6
Svendborg district	1 708	2 458	+ 43.9
Aalborg district	2 284	3 430	+ 50.3
Aarhus district	3 214	4 147	+ 29.0
Hjørring district	1 158	2 233	+ 92.8
Randers district	2 087	3 385	+ 62.2
Ribe district	2 086	2 914	+ 39.7
Ringkøbing district	2 308	3 645	+ 57.9
Skanderborg district	2 827	2 740	— 3.1
Thisted district	891	1 308	+ 46.8
Vejle district	2 243	3 184	+ 41.5
Viborg district	1 720	2 120	+ 23.3
Aabenraa-Sønderborg district	1 355	1 474	+ 8.8
Haderslev district	959	1 090	+ 13.7
Tønder district	463	730	+ 57.6
TOTAL	54 065	74 884	+ 38.5

By November 1955, the number of prescriptions for narcotics had increased to 75,000 (i.e., by 38.5%), and it was decided to analyse the prescriptions again, although less extensively than in 1949. This was facilitated by the fact that since November 1949 all pharmacies in Denmark enter the prescriptions on a special form every time narcotic drugs in group I are dispensed. Such copies are forwarded through the county medical officer to the National Health Service and have become the basis of the analysis.

Comparing the numbers, expressed in percentage, of prescriptions issued during the two trial months, there is a wide variation, as is shown by an increase of 92.8% in Hjørring and a decrease of 3.1% in Skanderborg, but comparing the number of prescriptions per 100 inhabitants the figures have otherwise approximated the average for the whole country. However, the three districts of Svendborg, Vejle and Ribe, which in 1949 were representative of the total consumption of the country, now show an increase which is the same as the average increase for the whole country. These increases were approximately 44%, 41% and 40% respectively, and these districts are therefore examined separately below. The total number of prescriptions for them was 6,037 in 1949 and 8,556 in 1955, but in this connexion it is pointed out that since 1949 more drugs such as ketobemidone and dexamphetamine have been added to the list of drugs for which prescriptions require to be entered by the pharmacists on a special form as above. Still others, already existing in 1949, such as thebacon and hydrocodone, have also been added to this list since 1949. The position in November 1955 may be analysed as follows :

Prescriptions for thebacon and hydrocodone	1,029
Prescriptions for ketobemidone	951
Prescriptions for dexamphetamine	659
TOTAL	2,639

Deducting this total of prescriptions for the "added" drugs from the 1955 total mentioned above — i.e., 8,556 minus 2,639 — the remainder, 5,917, shows close agreement with the 1949 figure of 6,037. Other factors to be borne in mind are the increase in population in the three districts concerned [1] and the more widespread use of drugs by older

[1] 23,371 between 1945 and 1950—i.e., 4.7%, as compared with 5.8% for the country as a whole.

people in age groups which have increased more rapidly than those of the younger members of the population.[2]

A comparison between the volume of doses prescribed in 1949 and in 1955 has not proved feasible, although for some preparations there seems to have been a decrease in the volume of single prescriptions.

Table 2 gives the figures of the wholesale trade in narcotic drugs based on returns made to the Permanent Central Opium Board. These provide a very rough estimate of annual consumption and the variations over the seven-year period. Whilst there has been a considerable fall in the consumption of morphine, this is counterbalanced by an increase in the consumption of synthetic analgesics, especially pethidine. Methadone, which was not placed under control in Denmark until 1952, is not representative of the general trend, and ketobemidone, first used in 1952, has been consumed at a constant rate during the last three years.

The high rate of consumption of codeine is specially worthy of note, since it forms a very high proportion of all narcotic drugs consumed, but this is partly due to its widespread use in tablet form combined with mild analgesics and in mixtures containing other sedatives. Furthermore, codeine is widely used in cough medicines, the consumption of which is very high in Denmark because of the prevalence of infections of the respiratory system caused by the climatic conditions. A similar situation is found in Sweden and Finland. Although there is no treaty obligation requiring prescriptions for codeine, as a drug in group II under the international conventions, most codeine preparations require a prescription under national legislation; such prescriptions can be repeated only five times.

[2] The number of people aged 60 in the whole country increased from 506,451 to 572,459 during the period 1945-1950—i.e., an increase of 13%.

TABLE 2

Wholesale (in kilogrammes)

Year	Morphine	Cocaine	Oxycodone	Hydrocodone	Thebacon	Codeine	Ethylmorphine	Pethidine	Methadone	Ketobemidone
1949	121.1	16.1	3.6	8.9	5.2	1 085	125.9	100.3	—	—
1950	183.2	22.9	4.9	14.5	7.6	1 614	130.2	213.3	(0.2)	—
1951	120.6	19.9	4.4	14.0	5.8	1 340	83.9	209.6	(0.4)	—
1952	129.7	10.4	8.2	13.2	7.9	1 062	76.3	166.0	15.2	9.0
1953	87.7	11.7	4.1	16.9	11.7	1 317	88.9	196.0	25.6	20.3
1954	93.8	14.4	3.9	14.0	10.2	1 612	82.9	186.3	21.9	19.7
1955	85.0	11.8	3.7	9.5	6.6	1 617	97.8	214.9	17.8	22.7

The consumption of ethylmorphine (a drug in group II under the international conventions) has never been so low as during the last five years.

Even if it cannot be said with certainty that the total consumption of narcotic drugs in Denmark has not increased during the past six years, the prescriptions indicate even a decrease in consumption when the changes in the population figures are considered.

Using Dr. Wiingaard's grouping, the drugs are classified as follows :

I. Morphine group A
II. Morphine group B
III. Diacetylmorphine group
IV. Pethidine group
V. Methadone group
VI. Ketobemidone group
VII. Thebacon-hydrocodone group
VIII. Cocaine group
IX. Amphetamine group
X. Dexamphetamine group

One of the most important developments is the greater use of the potent synthetic analgesics in place of the opium alkaloids. Thus, prescriptions for drugs from groups I and II have decreased 50%, which is more than table 2 indicates; and, as mentioned above, it cannot be said that the rate of consumption is attributable to the volume of single prescriptions. For example : expressed as a percentage, the share of the total consumption of methadone has not changed since 1949, and ketobemidone preparations are distributed to an extent surpassed only by pethidine.

Although ketobemidone is thought to be as dangerous as diacetylmorphine, and although the Economic and Social Council has urged governments to prohibit the manufacture, import and export of ketobemidone, its salts, its preparations and preparations of its salts, Denmark's non-compliance is due to the fact that experience in Denmark shows that ketobemidone is at least not misused more than morphine or other synthetic analgesics. This may be due to the fact that the main ketobemidone preparations sold in Denmark contain only one part of ketobemidone to five parts of " A 29 " which is 1,1-diphenyl-3-dimethylaminobutene-1. There is a possibility that the atropine-like effect of large doses of " A 29 " is so disturbing that the addiction-producing effect of ketobemidone is compromised.

The number of prescriptions containing diacetylmorphine has been reduced very much since 1949, so that this drug is now practically exclusively used as a low-concentrated ingredient of cough medicine and has almost everywhere been replaced by codeine and ethylmorphine. Prescriptions for cocaine have also been considerably reduced, probably as a result of the increasing use of synthetic anaesthetics.

TABLE 3

Group	Number of prescriptions in 1955				1949
	Magistral	Officinal	Total	%	%
I. Morphine A	184	170	354	4.1	8.7
II. Morphine B	181	1 194	1 375	15.9	32.6
III. Diacetylmorphine	41	10	51	0.6	2.2
IV. Pethidine	62	1 292	1 354	15.7	13.1
V. Methadone	12	834	846	9.8	9.8
VI. Ketobemidone	1	950	951	11.0	—
VII. Acetyldihydrocodeinone-hydrocodone	104	925	1 029	11.9	—
VIII. Cocaine	73	5	78	0.9	2.5
IX. Amphetamine	360	808	1 168	13.5	17.8
X. Dexamphetamine	0	659	659	7.5	—
Remainder group			785	9.1	13.3
			8 650	100.0	100.0

A moderate decrease in the number of prescriptions under the amphetamine group is counterbalanced by the additional prescriptions for dexamphetamine. Drugs in this group are not subject to the obligations of the international narcotic convention, but in Denmark prescriptions for amphetamine and dexamphetamine are subject to the same restrictions as those for group I narcotics — that is, they cannot be repeated, and have to be entered on a special form.

During the period 1949 to 1955, the drugs in the groups shown in the above table have changed as follows (the figure for 1949 is given in parentheses) :

Morphine groups A and B : 20% (40%)

Amphetamine-dexamphetamine : a little more than 20% (a little less than 20 %)

Pethidine : ca. 15% (13.1 %)

Methadone : ca. 10% (unchanged)

Ketobemidone : a little more than 10% (0)

Thebacon-hydrocodone : a little more than 10% (not liable to be repeated)

Diacetylmorphine and cocaine : each under 1% (each ca. 2%)

The remainder group : ca. 10% (ca. 13%)

Table 4 shows the prescription frequency for single preparations and, for purposes of comparison, ten of the most frequently prescribed preparations during 1949.

TABLE 4

1949	
Preparation	%
1. Tetrapon (mixed alkaloids of opium)	16.6
2. Pethidine	13.0
3. Mecodrine	11.7
4. gtt. Roseae (contains morphine)	10.1
5. Butalgin (methadone)	9.8
6. Morphine HCl	8.3
7. Amphetamine	6.3
8. Thebaica (raw opium)	4.5
9. Oxykon (oxycodone)	2.5
10. Cocaine	2.5

1955		
Preparation	*Number of prescriptions*	%
1. Pethidine	1 354	15.7
2. Ketogan (preparation containing ketobemidone)	923	10.7
3. Tetrapon (mixed alkaloids of opium)	724	8.4
4. Butalgin (methadone)	723	8.4
5. Dexedrine	659	7.5
6. Hydrokon (hydrocodone)	656	7.5
7. Amphetamine	571	6.6
8. Mecodrin (amphetamine)	486	5.6
9. gtt. Roseae (morphine solution)	373	4.3
10. Thebacon	350	4.0
11. Morphine HCl	345	
12. Opium	230	
13. Nirvapon (mixed alkaloids of opium)	177	
14. Scopolamin c. tetrapono (containing mixed alkaloids of opium)	145	
15. Opiopapaverini comp. (contains opium)	132	
16. Methadone	123	
17. Oxykon (oxycodone)	108	
18. Cocaine	78	
19. Algospasmin (contains mixed alkaloids of opium)	76	
20. Methylamphetamine	75	
21. Heroin comp. (contains diacetylmorphine)	50	
22. Opiatae comp. (contains opium)	48	
23. Algophon c. scop. (contains mixed alkaloids of opium)	48	
24. Pantopon (mixed alkaloids of opium)	38	
25. Cliradon (ketobemidone)	28	
26. Chloralico-morphine	26	
27. Eufodrin	23	
28. Cynoglossi (contains opium)	18	
29. Acedicon (thebacon)	17	
30. Kemodrin	13	
31. Hydromorphone	9	
32. Morf. citr. mit. (contains morphine citrate)	8	
33. Dicodid (hydrocodone)	6	
34. Dilaudid (hydromorphone)	5	
35. Dolantin (pethidine)	3	
36. Bonain (contains cocaine)	2	
37. Diacetylmorphine	1	
38. Dromoran (racemorphan)	1	
39. Morf. citr. fort. (contains morphine citrate)	1	

Table 5 shows that the prescriptions grouped according to recipients are practically unchanged since 1949.

TABLE 5

The recipients

	Total	%	1949 %
Patients	7 835	90.58	90.89
Animals	45	0.52	0.86
To be used in practice	463	5.35	5.21
To be distributed	55	0.64	1.05
Hospitals, etc.	245	2.83	1.90
For ships' stores	7	0.08	0.05
Unexplained	0	—	0.04
	8 650	100.00	100.00

Conclusion and Comments

Available data indicate that there is no basis for the assertion that the rate of consumption of narcotic drugs in Denmark in the period under examination shows an increasing tendency; on the one hand, consumption of morphine is decreasing, while, on the other, that of the synthetic analgesics is increasing. The consumption of the latter now seems more stable, although a further increase might have been expected owing to population growth and the high proportion of older people.

Nevertheless, the consumption rate in Denmark, even when compared with those of other Scandinavian countries, continues to be high; and in respect of morphine,[3] codeine, pethidine and methadone the figures for Denmark per million inhabitants in 1954 were the highest, with the sole exception of Iceland, where pethidine consumption was exceptionally high.

Factors to be borne in mind when considering the high consumption rate are :

(1) The Danish Health Insurance System covers nearly the whole population so that the sick have no economic difficulties in getting analgesics.

(2) Narcotic drugs are given only on doctors' prescriptions, and subject to the additional safeguards described above.

(3) No cases of illicit production or import of narcotic drugs have been reported.[4]

Although realizing that the high rate of consumption carries a risk of widespread misuse, the Danish health authorities believe that this risk cannot be completely eliminated, and it is expected that the rate will remain high as long as (1) the social-medical service has the same structure and extent as it has now; (2) the frequency of painful diseases does not markedly decrease; (3) the increase in population is domi-

[3] However, according to the 1955 statistics, the rate of consumption of morphine in Norway in 1955 was treble that in Denmark.

[4] Only very small quantities of narcotic drugs for injection are coming on to the illicit market.

TABLE 6

Consumption in kg per million inhabitants

		Denmark	Finland	Iceland	Norway	Sweden
Morphine	1946	33.41	25.54	22.73	12.24	9.23
	1950	42.72	15.35	14.18	31.39	9.88
	1954	21.06	6.44	6.49	18.87	6.24
Diacetylmorphine	1946	0.98	25.54	—	—	2.83
	1950	0.47	5.2	—	—	2.29
	1954	0.22	2.39	—	—	0.28
Codeine	1946	285.05	94.4	242.42	81.16	114.75
	1950	371.85	141.83	212.77	68.33	142.43
	1954	361.19	202.29	103.9	65.45	239.29
Ethylmorphine	1946	39.5	6.45	15.15	9.98	18.01
	1950	26.38	20.54	(1953)*	5.85	35.36
	1954	18.37	26.72	6.76	4.72	43.39
Cocaine	1946	3.41	1.03	7.58	9.02	2.68
	1950	5.37	2.72	21.28	3.08	2.58
	1954	3.14	1.43	6.49	1.47	1.25
Pethidine	1946	—	—	—	—	—
		(1951)	(1951)	(1951)		
	1950	47.86	3.24	13.98	8.62	1.15
	1954	41.68	2.86	71.43	12.09	1.11
Methadone	1946	—	—	—	—	—
		(1952)	(1951)		(1951)	(1952)
		3.44	2.99	—	0.61	0.28
	1954	4.93	3.82	—	0.29	1.25

nated by the absolute and relative increase in the old-age group compared with that in the average population; (4) the combating of pain by means of medicine can take place only with the help of dangerous drugs.

Legislative measures lately enacted to control narcotic drugs are :

1949 : Amendment to registration regulations so that liability to register includes drugs brought under group I control under the international narcotics conventions and, in addition, amphetamine preparations and compounds.

Furthermore, as mentioned above, in place of registers of prescriptions kept permanently in the pharmacies, pharmacies now make up a list of exact and complete copies of the prescriptions on special forms, which are forwarded to the National Health Service each month to be used as the basis for a card index which serves as a control of doctors' prescriptions and patients' consumption.

1955 : The Medical Act was made wider to give the National Health Service added authority to take measures against doctors who prescribe narcotic drugs in an unwarrantable way. Doctors became entitled to renounce their right to prescribe narcotic drugs, and the National Health Service to deprive them of that right.

Denmark's 353 pharmacies are being kept currently informed as to the names of doctors who have lost their right to issue prescriptions for narcotic drugs. Forty-two of Denmark's 5,500 doctors have so far lost this right, and one of the 1,600 veterinarians, but none of the 1,400 dentists. As far as the doctors are concerned the unjustifiable prescriptions of narcotics have in most cases been for their own use and not for that of their patients.

It should likewise be emphasized that most of Denmark's drug addicts are people who suffer from serious illnesses accompanied by severe pain which can be relieved only by the use of narcotics.

Finally, it may be noted that the card index, established in 1949, relating to the consumption of narcotic drugs now contains material which, scientifically processed, may throw a better light on the quantitative use and misuse of narcotic drugs than has hitherto been possible. As these investigations are finished, they will be made available to the international agencies.

Chasing the dragon
The smoking of heroin in Hong Kong

Information transmitted for publication by the Government of the United Kingdom; furnished by the Government of Hong Kong

The expression " chasing the dragon " is now a widely known and established idiom used in narcotic circles in this colony in connexion with the smoking of heroin. In those parts of the world which, like Hong Kong, suffer from a heroin addiction problem, the popular form of absorbing this type of drug is by means of injection. The hypodermic syringe is not a common feature of local heroin traffic, and our addicts on the whole continue to be shy of the needle. In Hong Kong, where heroin has now superseded opium as the main drug of addiction, the traditional form of smoking has been retained by addicts who indulge in the use of this drug.

Narcotic retailers normally sell very crude heroin in small plain or coloured cellophane packets costing H K $0.50, $1 or $2. The average weight of the powder in these packets is approximately 0.032, 0.063 and 0.126 g respectively. The cheapness of this commodity indicates that the heroin being sold in this form is of a crude quality and is therefore unfit for injection purposes. The large majority of addicts in Hong Kong cannot afford to purchase high-grade heroin, and to overcome this economic factor a method known as " chasing the dragon " has been adopted to satisfy their needs.

Fine heroin powder has a tendency to run into a single mass when heat is applied. The outside of this mass then becomes charred and tends to protect the inner portion of the mass from heat, thus preventing complete combustion, but naturally resulting in a certain wastage. For smoking, therefore, crude heroin in rough granular form is preferred by the local heroin addicts and is available in packages as outlined above. With each purchase, the retailers also issue a base powder commonly known as " daai fan ". This is generally one of the barbiturates, usually also in grain form, the purpose of which is to prolong the effects of the heroin and to aid sublimation of the heroin when heat is applied.

The implements required for the purpose of smoking consist of a piece of tinfoil of the size found in the average cigarette packet, a paper tube similar to a drinking straw, some cardboard spills and a small lamp. The tinfoil is first smoothed out with a bone or metal instrument until it attains a high polish. It is then folded in half and the heroin and base powder are placed in the crease about half an inch apart. A cardboard spill is then lit and the flame is applied to the tinfoil directly underneath the base powder which liquefies and mixes with the heroin. The tinfoil is then tilted back and forth whilst the flame is applied directly under the crease; at the same time the smoker directs the paper tube which is held in his mouth over the tinfoil and inhales the fumes emanating from the heated mixture.

To obtain the best effect the paper pipe chases the " smoky tail " of the liquid which is moving back and forth in the tinfoil trough until nothing is left on the tinfoil except a black stain. With the usual local aptitude for a turn of phrase this whole procedure has been called " chui lung " or " chasing the dragon ".

This simple method of consuming heroin dispenses with pipes and other paraphernalia normally utilized in the smoking of opium and heroin pills. As the minute quantities of crude heroin and base powder are cheap, easy to conceal or throw away, the chances of the addict's being arrested with incriminating evidence in his possession are greatly lessened.

A pharmacological investigation and evaluation of the effects of combined barbiturate and heroin inhalation by addicts who employ this method of smoking heroin has been specially made in connexion with the preparation of this article by Dr. Carl C. Gruhzit, M.D., Ph.D., Lecturer in Pharmacology in the University of Hong Kong.[1]

[1] See next article.

This photograph shows the materials necessary for smoking heroin. The man is holding in his hands a folded piece of tinfoil containing a mixture of heroin and base powder. The cardboard spill which he uses to heat the mixture is held between the fingers of his right hand, and the paper tube through which the smoke is inhaled is held in his mouth. The lamp on the table is used to light the spills. To the right of the lamp may be seen two small pieces of cellophane paper. One holds heroin, the other " daai fan ".

This photograph shows, in the left foreground, a piece of tinfoil containing a mixture of " daai fan " and heroin. The man on the right is cutting up cigarette packets for use as spills. In front of him may be seen a tinfoil trough containing a large blob of heroin and base powder already mixed and liquefied. Farther along the table may be seen two of the paper tubes used to inhale the fumes of the drug.

Pharmacological investigation and evaluation of the effects of combined barbiturate and heroin inhalation by addicts [1]

By Dr. Carl C. Gruhzit, M.D., Ph.D.,
Lecturer in Pharmacology, University of Hong Kong

Initial observations of addicts who employ barbiturates as a base powder during the smoking of heroin suggested that some of these individuals might suffer from the deleterious effects of chronic barbiturate intake, or in fact be addicted to both barbiturates and heroin. Therefore, an investigation was undertaken to determine (1) the nature of the base powder, (2) the amount of base powder used by addicts, (3) the effect of volatilization of the base powder and (4) the amount of volatilized base powder inhaled by the addicts. On the basis of these results, previous published information and clinical observations, an evaluation has been made of the effects of combined barbiturate and heroin inhalation in addicts who " chase the dragon ".

Analysis of Substances used by Addicts who " Chase the Dragon "

Four samples of base powder or " daai fan " were chemically tested and assayed according to the procedure in the British Pharmacopoeia (1953). Relatively pure sodium barbitone powder (May & Baker) was employed as a control of the methods of identification and assay. All samples of base powder were identified chemically as barbitone base and assayed at 97 $\pm$ S.D. 3%. It is suspected that the colouring matter employed in at least one of the samples was litmus, but this matter was not investigated further (appendix 1).

The base powder (dissolved in weak alkali) was tested biologically in eight rabbits, and the results were compared with those produced by the administration of sodium barbitone. The effects produced by the administration of base powder were indistinguishable from those produced by the administration of an equivalent amount of sodium barbitone (appendix 2).

Twelve packets of heroin tested according to the method in the British Pharmacopoeia were determined to contain diacetylmorphine hydrochloride. The melting-points ranged between 222°C and 229°C indicating that the samples were not highly purified. Assay of the samples indicated that they contained 92 $\pm$ S.D. 5% heroin hydrochloride (appendix 3).

These results obtained by chemical and biological assay are in close agreement with the findings of the government chemists, except that on occasion they have found phenobarbitone employed as the base powder. Since barbitone and phenobarbitone have similar pharmacological effects, the results with barbitone are applicable to both drugs.

[1] See preceding article.

Volatilization and Condensation of the Base Powder

Twenty-seven one-gramme samples of the base powder were volatilized by gentle heating. The vapour was collected and condensed by a cooling jacket and the condensate was dissolved in warm alcohol. The alcoholic solution was evaporated to dryness and the residue was weighed and tested chemically and biologically for activity. Following volatilization, 57-95% of the barbitone could be recovered (appendix 4). The degree of recovery was primarily dependent on the nature of heating. Very gentle heat gave high recovery yields, while stronger heat resulted in partial decomposition of barbitone to carbon, carbon dioxide, ammonia and other unidentified by-products. The residue left in the flask after heating formed a black stain similar to that observed on the tinfoil used by addicts.

Biological testing of the material collected after volatilization produced results which were in close agreement with those obtained by chemical assay (appendix 5).

The time required by addicts to volatilize the combined base-powder-heroin mixture and the formation of the residue on the tinfoil indicate that a significant, but not excessive, portion of the base powder used by addicts is decomposed. Although no actual data can be obtained, it is estimated, on the basis of these observations, that approximately 15-25% of the base powder is broken down by heating during the process of " chasing the dragon ". The exact amount which is decomposed will certainly vary with the habits of the individuals.

Estimation of the Amount of Heroin and Barbiturates inhaled by Addicts

It is naturally difficult to obtain information regarding the actual intake of base powder by addicts. The base powder is freely available and, in general, can be used in any quantity which the smoker desires. Observation of the addicts during the process of " chasing the dragon " is, in general, unsatisfactory, because under surveillance they avoid the use of heroin. Estimates by addicts (prisoners), however, indicate that they usually use about four granules of base powder per granule of heroin. This is approximately the ratio utilized when heroin was given to addicts for observational purposes. This is also the approximate ratio of base powder and heroin contained in " prepared packets " sold to some addicts.

The average addict spends approximately HK $3-4 per day on heroin, and an occasional addict spends more than HK $10 per day on heroin. At the current prices, this is the equivalent of the smoking of approximately 0.2-0.25 g of heroin per day by the average addict and more than 0.75 g per day by the addict with a large consumption. If only 50% of the fumes are inhaled, the average addict inhales over 0.1 g of heroin per day, and some individuals take in over 0.3 g per day.

If one assumes that the addict who " chases the dragon " uses four times as much base powder as heroin, the average addict would use about 1.0 g per day of barbitone. Depending on the amount of decomposition with heating, and the skill of the addict in inhaling the fumes, the average total intake would probably be only 50-75% of this amount. However, the addict who consumes large quantities of heroin also uses large quantities of base powder, and under these conditions the daily intake of base powder may exceed 1.5 g.

These estimates of drug intake are confirmed by clinical observations *(vide infra)* and results of the determination of barbiturate levels in the blood and urine specimens obtained from newly admitted prisoners who " chased the dragon " just prior to their arrest (appendix 6).[2]

Evaluation of the Effect of Drug Intake

The results of controlled studies and the study of the withdrawal symptoms induced by the administration of nalorphine indicate that the daily intake of even less than 0.02-0.04 g of heroin may produce addiction. Even the least adroit person who " chases the dragon " probably inhales far in excess of this amount. *Therefore, an individual who " chases the dragon " repeatedly will become addicted to heroin.* This fact is confirmed by observing individuals at liberty and by the medical examination of individuals committed to prison. One can detect the addicts by their characteristic withdrawal symptoms even prior to questioning. Even the addicts who claim to smoke only one to two HK dollars' worth of heroin per day show definite withdrawal signs.

The addicting dose of barbiturates is also variable. However, controlled studies and clinical observations indicate that the continued intake of 0.6-0.8 g per day of the short-acting barbiturates — e.g., pentobarbitone — produces addiction in the majority of individuals. The addicting dose of the barbiturates varies with the potency of the compound, and therefore larger amounts of barbitone would be required to produce addiction. *The total intake of barbitone by the average individual who " chases the dragon " is not sufficient to produce barbiturate addiction. Individuals who consume large quantities of heroin, and therefore consume large quantities of barbitone are, however, likely to become addicted to barbiturates.* Addiction is also likely to occur in individuals with renal disease who have impaired excretion of barbitone. It is also probable that many individuals who have insufficient funds to purchase heroin use barbitone as a substitute and thus become addicted to the latter drug. *Even non-addicting* amounts of barbitone would be likely to produce some degree of sedation, and in higher doses, mental confusion, a staggering gait, and slurred speech.

This theoretical evaluation based on the estimate of drug intake is adequately confirmed by observing addicts and individuals during their withdrawal period. The majority of heroin addicts do not show signs of chronic barbiturate intoxication. Nevertheless individuals have been observed who do show signs of barbiturate addiction *(vide supra)*. Unfortunately, severe heroin addiction may also produce similar signs, and it is difficult to attribute the correct proportion of signs to each of the two drugs.

The majority of individuals who cease " chasing the dragon " (because of incarceration) *do not show typical signs of barbiturate withdrawal.* There is no skin rash (which may result from chronic barbiturate administration), nystagmus or tremors in most addicts during withdrawal. Nevertheless, a significant percentage (about 1%) of prisoners who formerly " chased the dragon " develop convulsions during the withdrawal phase. These convulsions are not usually produced in individuals addicted to the opium alkaloids or their derivatives, but are characteristic of the withdrawal signs in individuals severely addicted to barbiturates. The percentage of individuals showing convulsions probably represents only a minimal figure for barbiturate addiction. It is likely that many individuals who have only tremors or minor twitches during their withdrawal period are not observed during their attacks and are therefore not reported to the medical officer. *It is therefore likely that at least about 5-10% of individuals who " chase the dragon " have combined heroin and barbiturate addiction.*

In many respects barbiturate addiction is a greater danger than addiction to the " narcotic " group of drugs. The depression of mental facilities which it produces is usually greater than one observes in " straight " morphine or heroin addicts. In addition, the effects produced by barbiturate withdrawal are considered by most workers in the field to be more severe and more likely to cause death than those produced following " narcotic " withdrawal. In fact, the withdrawal signs may be so severe that from a medical standpoint abrupt withdrawal is counterindicated in any individual who is suspected of being a barbiturate addict. Certainly the relatively high incidence of convulsions which has been observed in prisoners indicates the wisdom of this advice.

The combined use of heroin and barbiturates produces a particularly odious form of drug addiction. The central nervous system depression produced by these drugs is additive and may result in marked stupor. Furthermore, the individual has physical dependence on two distinct types of depressants, and continued intake of both drugs is necessary to prevent withdrawal symptoms. From a medical standpoint the combined use of heroin and barbiturates represents a great danger to the individual.

[2] The blood and urine samples where obtained from prisoners by Dr. C. O. Lee and were analysed by Dr. E. G. N. Greaves, Government Chemist.

APPENDICES

Appendix 1

Four samples of base powder were subjected to the following chemical tests for the identification of barbitone. The results obtained were identical to those obtained using known samples of barbitone base.

1. Solubility in water, 95% alcohol, solvent ether, chloroform and alkali hydroxides.
2. A gelatinous precipitate was produced when several drops of mercury nitrate solution were added to a saturated aqueous solution of the test substance acidified with nitric acid.
3. The addition of 1 ml of solution of copper sulphate with pyridine to a solution containing 0.1 g of the test substance dissolved in 1.5 ml of N/1 sodium hydroxide and 10 ml of 10% w/v pyridine in water produced a lilac-coloured precipitate.
4. Fusion of the test substance with caustic alkali or boiling of a strong caustic alkali solution of the test substance resulted in the production of ammonia.

Melting-point determinations on the base powder ranged between 185°C and 191°C. Melting-point determinations on known samples of barbitone base ranged between 190°C and 192°C.

Base powder	Test 1	Test 2	Test 3	Average
1.	187	185	188	187
2.	188	190	185	188
3.	190	191	186	189
4.	188	185	188	187
			Average ..	188
Barbitone base ..	192	190	190	191

Assay of the base powder showed the following percentage barbitone content :

Base powder	Test 1	Test 2	Test 3	Average
1.	102	94	97	97.7%
2.	95	101	96	97.3%
3.	100	93	93	95.3%
4.	98	102	97	99.0%
			Average ..	97% ± S.D. 3%
Sodium barbitone	103	100	101	101.3%

Appendix 2

The intraperitoneal injection in rabbits of 200 mgm/kgm base powder (dissolved in weak alkali) produced effects which were indistinguishable from those produced by the injection of an equivalent amount of sodium barbitone (calculated as base).

Rabbit	Base powder	Latent period of anaesthesia (Minutes)	Duration of anaesthesia
1	1	38	490
2	1	27	330
3	2	33	380
4	2	48	460
5	3	46	420
6	3	29	360
7	4	37	320
8	4	39	370
	Average ..	37	390

Comparable experiments employing sodium barbitone gave an average latent period of 34.5 min. and a duration of anesthesia of 420 min.

Appendix 3

The following chemical tests were used to confirm that twelve packets of " heroin " contained diacetylmorphine hydrochloride :

1. Solubility in water, 90% alcohol and solvent ether.
2. Contact with nitric acid produced a yellow colour which changed to bluish-green on heating.
3. Heating following the addition of 1 ml of sulphuric acid to a 2 ml solution of 90% alcohol in which 0.1 g of heroin was dissolved produced the characteristic odour of ethyl acetate.
4. The samples gave positive tests for chlorides.
5. The yellow colour produced by the addition of 2 ml of 1% w/v sodium nitrate in water followed by the addition of 3 ml dilute ammonium hydroxide to 0.5 ml of 0.1% w/v heroin solution in N/10 hydrochloric acid was less than when 0.0015% w/v anhydrous morphine in N/10 hydrochloric acid was treated in a similar fashion.

As shown in the following table, the melting-point determinations of the heroin samples ranged between 222°C and 229°C. The melting-point of pure diacetylmorphine hydrochloride is listed at 229-233°C. Because of the size of the packets it was necessary to use different samples of heroin for identification tests, melting-point determinations and assay procedures.

Packet	Test 1	Test 2 (Degrees Centigrade)	Average
13	226	223	224.5
14	222	224	223
15	229	227	228
16	224	223	224.5
17	227	223	225

Uncontrolled assay of samples of heroin indicated that they contained 92% ±S.D. 5% diacetylmorphine hydrochloride. The packet size did not allow for duplicate determinations to evaluate the variations in the test methods. It is likely that the large standard deviation is due in part to variations in the testing and in part to variations in the samples tested.

Packet	% diacetylmorphine hydrochloride content	Packet	% diactylmorphine hydrochloride content
18	93	24	99
19	84	25	80
20	88	26	93
21	87	27	88
22	96	28	95
23	92	29	93

Appendix 4

One-gramme samples of base powder were volatilized and the vapours were collected and condensed by a cooling jacket. The condensate was dissolved in warm alcohol and evaporated to dryness, and the residue was weighed and tested chemically and biologically for activity.

Sample	Volatilization time (Minutes)	Recovery %
1	22	92
2	18	97
3	17	90
4	15	82
5	15	80
6	13	83
7	12	79
8	11	78
9	11	86
10	11	77
11	11	74
12	10	82

Sample	Volatilization time (Minutes)	Recovery %
13	10	84
14	10	78
15	10	69
16	10	78
17	10	78
18	10	74
19	9	68
20	9	71
21	8	69
22	7	68
23	7	59
24	5	66
25	5	62
26	5	57
27	5	57

Appendix 5

The intraperitoneal injection in rabbits of 200 mgm/kgm of the condensate (dissolved in weak alkali) obtained after volatilization of the base powder produced results comparable to those obtained following the injection of an equivalent amount of sodium barbitone (dose calculated as base).

Sample	Latent period of anaesthesia (Minutes)	Duration of anaesthesia (Minutes)
15	33	370
16	44	380
17	30	420
18	37	430
Average ...	36	400

Appendix 6

Blood and urine specimens were obtained from each of two prisoners by Dr. C. O. Lee and were analysed by Dr. E. G. N. Greaves, Government Chemist. Both patients had been deprived of drugs for three to five days prior to the sampling. Barbiturates were not detected in one patient, who, it is estimated, inhaled only 0.3-0.4 g of barbitone per day. The majority of barbitone is excreted within 48-72 hours following cessation of drug intake, and in this individual presumably only undetectable traces remained in the blood and urine at the time of sampling. In the second patient significant amounts of barbitone were detected in the blood, indicating a high level of barbiturate intake, which is compatible with the relatively large amounts of money which this patient spent on " chasing the dragon " prior to his arrest.

Although technical difficulties prevent an accurate quantitative determination of the blood and urine barbitone level in the second patient, it is likely that he was inhaling sufficient barbitone to develop combined heroin and barbiturate addiction as the result of " chasing the dragon ".

Opium prohibition campaign in Assam [1]

By Shri E. H. Pakyntein, I.A.S.
Excise Commissioner, Assam

The opium question in the State of Assam, one of the constituent states of the Republic of India, has been of considerable official and public interest in India, particularly during the years 1920 to 1940. In some measure, the policy adopted in that state regarding opium consumption was considered by some as the forerunner of the policy later applied all over India. The present article reviews the development of the measures taken in Assam. A comprehensive review of the policy of the Government of India regarding opium consumption may be found in the article entitled "Abolition of opium smoking in India" published in the Bulletin of Narcotics, *Vol. IX, No. 3.*

Assam was known in the past as the "black spot" of India, owing to the heavy consumption of opium there, both oral and smoking. Opium had in fact been the bane of the economic and social life of the Assamese people. It appears from history that opium was a close preserve of the Ahom royal family during the Ahom rule of Assam. The habit was gradually acquired by the nobility and high court officials, and eventually it became universal. Poppy cultivation was no doubt extensively carried on throughout the land. When Assam was annexed by the British as a result of the Treaty of Yandaboo in 1826, the position remained much the same. The then administration at once found a source of income from opium, and lost no time in making the opium trade a state monopoly. Steps were also taken to suppress the cultivation of poppy by the peasants, solely with a view to advancing the interests of government revenue. Poppy cultivation was so freely and extensively carried out that government measures to suppress it met with stiff opposition everywhere, culminating in the "poppy revolution" at Phulaguri in the district of Nowgong in the late 'nineties. The rebellion was put down with a firm hand, but after casualties on both sides. After this, the Government continued its monopoly by auctioning off the opium shops. Until the year 1919, there were no effective restrictions on the sale and consumption of opium. Till then, the policy was to lessen consumption by raising the treasury price, reducing the number of opium shops, restricting the limit of private possession and raising the permitted age of consumers. In spite of this policy, the guiding factor was revenue and not the moral, social and economic uplift of the people. During 1918/19, the total sale of opium stood at 1,324 maunds 26 seers,[2] yielding a revenue of Rs. 3,391,522.

The year 1919 marks the beginning of some policy of opium prohibition. To begin with, the system of registration of consumers was introduced in the Sadiya frontier tracts. The system was extended in the following years to other areas known as "backward areas". In 1920/21, shop rationing, on a gradually descending scale, was introduced in the plains districts of the state. The Assam Legislative Council, in its session in March 1921, passed a resolution to the effect that the opium consumers should be registered, and each consumer should be given a definite quota of ration and that the rations should be reduced by 10% annually, so that in ten years the rations would be abolished save on medical grounds. Registration of consumers was introduced on an experimental basis in the Goalpara district in 1924. The system was gradually extended to the other plains districts, and by 1927 the system of registration of consumers and rationing was in full force throughout the state. In 1927, the Legislative Council passed a resolution recommending gradual reduction of the ration of consumers below the age of fifty years with a view to bringing it down to nil within ten years. The resolution was accepted by the Government, and positive steps were taken from 1 April 1927 onwards to give effect to the resolution by carrying out the first 10% cut. In 1927/28, there were about 98,000 registered opium consumers with an annual ration of 792 maunds, and yielding a revenue of Rs. 3,825,730. Registration of consumers and accelerated reductions of ration of consumers below the age of fifty continued to be in force until 1933. In 1933, the Assam Legislative Council passed a resolution to appoint an inquiry committee to review the results of the then opium policy and to advise on the future course of action. An inquiry committee was accordingly appointed, and the committee submitted its recommendations. The policy of the Government, adopted on the recommendations of this committee, was to continue the rationing and the reduction of ration, and not to issue any new ration cards on grounds of addiction. These measures to check the consumption of opium remained in force until 1937. In 1937, an excise committee consisting of some members of the Legislative Assembly was constituted and, as a result of its recommendations, it was decided that no new ration card should be granted or the ration of the existing consumers raised without specific orders from the Government. Further, the powers of the deputy commissioners to issue new ration cards were resumed by the Government. By 1938/39, the tenth (or the last) percentage cut was carried out; and at the close of this year, the number of registered consumers stood at 30,366, with a ration of 180 maunds. Between 1927/28 and 1938/39, 67,634 ration cards with a ration of 612 maunds thus disappeared. This disappearance was not only due to the reduction of ration, but also to such causes as deaths and transfer of residence, etc. It would appear that public

[1] See *Bulletin on Narcotics*, Vol. IX, No. 1, in relation to the problem in the whole of India.

[2] 1 maund = 37 kg 312 g, 1 seer = 932.8 g.

opinion in Assam was definitely against the opium habit since 1921. Public opinion found a true expression when, on 15 April 1939, the Congress Coalition Government of Assam took a decisive and bold step by introducing the scheme of total prohibition of opium in the two sub-divisions of Dibrugarh and Sibsagar, which had the heaviest addiction. On this date, 10,150 ration cards with an annual ration of 61 maunds for these two areas were cancelled, and 61 opium shops were closed down. With this cancellation, the number of registered consumers came down to 20,216, and their ration to 119 maunds. A non-official organization was associated with the prohibition campaign in order to supplement the official organization, but the former did not continue long. The system of total prohibition in the two sub-divisions mentioned above, and of reduction of ration in other areas, continued to be in force between 1939 and 1941. Total prohibition was extended to cover other areas of the plains districts between 1941 and 1948. The non-official organization was revived in 1946/47 in order to campaign for total prohibition and to gain control over the smuggling of contraband opium. To achieve proper control over opium smuggling, Assam went ahead of other states by passing the Assam Opium Prohibition Act in 1947. The Act was promulgated on 1 April 1948. This Act gave legal sanction to the non-official organization. The Act provides for heavy compulsory sentences of fine and imprisonment, enhanced sentences for habitual offenders, restriction of movement, and also banning of known and notorious smugglers. The number of registered consumers stood at 2,661, with an annual ration of only 11 maunds on 1 April 1948. This number represented such ration cards as were granted and continued to be valid on medical grounds.

In the September 1948 session of the Assam Legislative Assembly, Shri Bijay Chandra Bhagabati, M.L.A., moved the following resolution :

> " That this assembly is of opinion that the Government of Assam do move the Government of India to help in making the Opium Prohibition Campaign in Assam a success by making poppy cultivation and traffic in opium illegal and punishable in law all over India, including the states, and to take such effective steps as to stop smuggling of opium into Assam from other provinces and countries."

In reply, the then Minister of Excise accepted the resolution, which was adopted by the House. In November 1948, a memorandum was submitted to the Government of India with certain recommendations in the light of the above resolution. An All-India Opium Conference was held in New Delhi on 3 August 1949,[3] and certain resolutions, — including one recommending total prohibition within a maximum period of ten years of the use of opium for purposes other than medical and scientific — were adopted. The objective is the total prohibition of consumption of opium by 31 March 1959. Assam's quota of opium as fixed by the Narcotics Commissioner will be nil by 31 March 1959.

In 1949, a survey of addicts who were getting opium from illicit sources was undertaken separately and independently by departmental and non-official agencies in certain districts. It was necessary because the campaign was against the addicts and also against the smugglers. The official agency estimated the number of unregistered addicts at 13,919, and their consumption at 94 maunds 18½ seers, whereas the non-official agency put these figures at 11,410, and 79 maunds 21 seers 16 tolas. Taking the average of these results, the number of unregistered addicts obtaining supply through illicit sources can roughly be put at 12,665, with an annual consumption of 87 maunds 4 seers 68 tolas.

The non-official organization devised a form for the survey which was filled in, after local inquiry, by the prohibition officers and non-official workers. The forms thus completed were passed on to the excise officers, who, in turn, checked the entries, making additions and alterations.

The success of total prohibition depends mainly on the elimination of possible new recruits. To this extent, our scheme has been considerably successful in that it has largely done away with the opium smoking assemblies of the past, thus making it possible to wean away the younger generation from the evil. The present methods of vigilance, propaganda and detection and prevention of smuggling of contraband opium cannot eliminate fresh addiction without first stopping the flow of contraband opium from outside the State of Assam. Of late, there has been a tremendous flow of opium from Kachin, between India and Burma. Prior to 1923/24, the smuggling of opium was mainly from Assam to Burma and elsewhere. The position was reversed in 1923/24. The Assam inquiry committee's report and facts and figures about opium in Assam indicate that Chinese and Tibetan opium has been coming into Assam since then through Burma and Bhutan, whereas Nepal, Malwa, Punjab, Rajasthan, northern India and Cooch-Behar opium poured in from the west. The west still continues to be the main source of supply of opium for the Assam smugglers.

The underground world of smugglers has very wide ramifications. The routes followed are diverse : by rail, river, air and through railway, steamer and postal parcels from other parts of India and by jungle paths through Burma. The methods adopted are very ingenious.

A large measure of success with total prohibition and within a reasonable time limit can, in the very difficult circumstances stated above, be achieved only by eliminating all possibilities of fresh addiction and bringing the addicts under strict control. The first depends on stopping the flow of contraband opium, and this can be achieved only by controlling the addicts and delivering them from the clutches of the smugglers. Once the possibilities of fresh addiction are eliminated and the control over existing addicts becomes effective, smuggling will automatically be stopped. The question, therefore, is how to control the opium addicts. In Assam, we have a register of all the addicts, and we are treating them all medically in treatment centres spread all over the state at considerable government expense. The results so far achieved are encouraging, and the system of treatment — which extends to two months — is continuing. In this way we hope to be able to

[3] See *Bulletin on Narcotics*, Vol. IX, No. 1.

complete treatment of the known 15,000 addicts in about two years.

It is necessary to point out here that the treatment of opium addicts to bring about a complete change in their nature, so that the craving for opium disappears, has been found to be successful. It cures the addicts within six weeks; but, to be on the safe side, they are kept for two months in the treatment centres. We cannot risk discharging the addicts earlier.

Assam looks to India and her sister state of West Bengal for co-operation and assistance in her final drive against opium, and is confident of getting it in abundant measure in the years to come, in the same manner as she used to get it in the years which have rolled by. It is our firm belief that opium in Assam will be a thing of the past before long.

The manufacture of codeine from morphine

By Walter R. Heumann, D.Sc.
Associate Professor of Chemistry, University of Montreal, Montreal, Canada

The methylation of morphine is one of the key operations of the opium alkaloid industry, as up to 90% of the manufactured morphine is converted into codeine, the methylether of the phenolic hydroxyl of the morphine. The latter is a rather precious raw material for a commercial-scale manufacture, and many attempts have been made in the course of almost one century first to realize and then to bring to perfection this phenolic methylation of the morphine. After discussing chronologically this development, the present paper describes a methylating method which can be seen to be its logical consequence.

In the chemical equations throughout this article, the abbreviated formulae M.OH and $M.OCH_3$ will be used for morphine and codeine respectively.

The first to achieve the methylation of morphine was Grimaux (5) in 1881, using methyl iodide as the methylating agent in alkaline solution :

$$(1)\ M.OH + CH_3I + KOH \longrightarrow M.OCH_3 + KI + H_2O$$

During the years following Grimaux's synthesis other classical methylating agents such as dimethyl sulfate or diazomethane were used to prepare codeine (7, 8). All these reactions had one important disadvantage — namely, that the nitrogen of the morphine reacted easily with one molecule of the methylating agent, forming quaternary ammonium compounds. These secondary reaction products are unstable and decompose easily, thus causing considerable losses of morphine. Numerous German patents (4) are evidence of the efforts made to overcome this drawback. A relatively satisfactory solution was found in 1909, when the German manufacturer C. H. Boehringer (1) introduced the use of methyl groups containing quaternary ammonium compounds as methylating agents. Such quaternaries cannot, of course, give the above-mentioned side reactions with the nitrogen of the morphine. It was thus that trimethylphenylammonium chloride was to become the outstanding methylating agent for morphine for many years. The reaction involved was described by the inventor as follows :

$$(2)\ M.OH + NaOH + (CH_3)_3C_6H_5N.Cl \longrightarrow M.OCH_3 + (CH_3)_2C_6H_5N + NaCl + H_2O$$

The reaction was carried out in ethanol solution, and it worked satisfactorily only at temperatures between 120°C and 130°C. The solution was therefore heated in an autoclave, whereby the pressure reached 4 to 5 atm. A detailed description of a commercial-scale application of this method was given by Schwyzer in 1927 (10). This author dissolved the morphine in an alcoholic solution of sodium ethoxide, added then the equivalent amount of trimethylphenylammonium chloride, and heated the autoclave until the pressure reached 4 atm. which was maintained for one hour. The methylation is not complete, and between 5% and 10% of the morphine can be recovered as such. The yield of codeine is approximately 90% of the theoretical equivalent of the morphine used up by the reaction. The loss of about 10% of morphine may be due to the rather crude conditions of temperature and pressure combined with both the strongly alkaline reaction of the mixture and the presence of water. It is known that morphine is easily destroyed by oxidation in alkaline aqueous solution.

A certain simplification of the process was a method first published by the British forces' intelligence reports on German technical processes after World War II (2). This method, which according to this report had been used by a German manufacturer since 1910, avoided the use of the autoclave as follows : The morphine was dissolved in a solution of potassium hydroxide in absolute ethanol. To this solution of potassium morphinate an equivalent amount of trimethylphenylammonium chloride is added. This mixture is then added slowly to xylene, which is kept at 125°C, whereby the alcohol distills off together with the water present. Only 2% of the morphine remains unchanged, according to the report, which, however, fails to mention the yield of codeine. That the methylation apparently goes further than in the autoclave seems to be due to the fact that the solution is immediately freed of water as soon as the temperature rises. As will be shown later, there is strong reason to believe that water has an inhibiting effect on the reaction.

A further attempt towards improvement was the use of the free trimethylphenylammonium base instead of its chloride, first described by Rodionov in 1926 (9). This author added the salt of the ammonium base to an alcoholic solution of sodium ethoxide and thus obtained a solution of the free base. The reaction is described by the author as follows :

$$(3)\ (CH_3)_3C_6H_5N.Cl + NaOH \longrightarrow (CH_3)_3C_6H_5NOH + NaCl$$

The sodium chloride precipitates and is filtered off. To this solution morphine is added, and the temperature is raised to 110°C, whereby the alcohol distils off. The author gives the following equation for the reaction :

$$(4)\ M.OH + HO.N(CH_3)_3C_6H_5 \longrightarrow M.OCH_3 + C_6H_5N(CH_3)_2 + H_2O$$

According to Rodionov — who described only laboratory experiments — about 20% of the morphine was recovered, the remaining 80% yielding approximately 85% of its theoretical equivalent of codeine.

It may be noticed that Rodionov used a solution of alkali ethoxide to prepare the free quaternary base, but gives an equation for the reaction with alkali hydroxide. Furthermore,

he obtained morphine by heating to 110°C only, instead of the usual 120°C to 130°C. The solution of his methylating agent was perhaps a mixture of the free quaternary base with its ethoxide, which could form as follows :

$$(5)\ (CH_3)_3C_6H_5N.Cl + Na.OC_2H_5 \longrightarrow (CH_3)_3C_6H_5NOC_2H_5 + NaCl$$

Such alkoxides of quaternary ammonium bases were first described by Ingold & Patel in 1933 (6). They had to be prepared under strictly anhydrous conditions, as they were found to be very easily decomposed by water. Rodionov's solution was prepared and handled without these precautions, and was therefore either a solution of the free quaternary base or the above mixture of free base and its ethoxide.

One German manufacturer, as reported by the British forces' intelligence service (3), obtained better yields of codeine with a certain modification of Rodionov's method. The solution of the free quaternary base is prepared by adding its hydrochloride to an alcoholic solution of potassium hydroxide, whereas Rodionov used sodium ethoxide. The morphine is then added slowly to xylene, which is kept at 125°C. The yield of codeine is reportedly 95%.

As shown by equations (2) and (4), the methylation has been described hitherto by one summary equation only. However, the reaction proceeds in fact in two distinct steps, as will be demonstrated by the new method described below. The morphine reacts first with the quaternary base to form the morphinate of the latter. This first step is an acid-base reaction involving an extremely weak acid, the morphine. Such reactions are difficult or even impossible to achieve in water, but they are favoured by solvents of lower dielectric constants, and could possibly become quantitative in solvents such as anhydrous alcohols, provided the formation of water by the reaction itself is avoided. This may explain why, in the methods cited, a certain amount of morphine remains unreacted. As will be shown later, this first step takes place at ordinary temperature. In the second step, the morphinate of the quaternary base splits into codeine and dimethylaniline by the action of heat.

It thus seems that water affects the methylation in two ways. As already pointed out, it may contribute to the oxidative destruction of morphine and thus lower the yield of codeine. On the other hand, it may also prevent the first step of the methylation from proceeding to completion. Water is introduced by using commercial morphine base, which always contains one molecule of water of crystallization, and also by the solvent ethanol, which is hygroscopic and absorbs up to 5% of water, when the reaction is carried out in contact with the atmosphere.

When the reaction is carried out according to equations (2) or (4), water is also formed in the first step as the product of neutralization. If, however, the free quaternary base or its salt were replaced by its ethoxide, ethanol would be formed instead of water, and the methylation would thus become easier. Following the line of these considerations, it appears logical to use the pure ethoxide of the quaternary base and to work under anhydrous conditions. Such a methylation method is now to be described here.

The ethoxide of trimethylphenylammonium can be prepared according to equation (5) by dissolving under strictly anhydrous conditions the hydrochloride of the quaternary base in a solution of sodium ethoxide in absolute ethanol at a temperature not exceeding 14°C. The sodium chloride which crystallizes is separated by decantation or vacuum filtration. The resulting clear light-brown solution of the quaternary ethoxide is stable for at least several days when protected from humidity and stored at or below 14°C. Its concentration is determined immediately before use by dissolving an exactly weighed sample in at least ten times its weight of water and titrating with 0.1 N standard acid.

Anhydrous morphine base dissolves readily in this cold solution to form the morphinate of the quaternary base. This is the first step of the methylation, which can be presented as follows :

$$(6)\ M.OH + C_2H_5O.N(CH_3)_3C_6H_5 \longrightarrow M.ON(CH_3)_3C_6H_5 + C_2H_5OH$$

When this solution is heated up to 110°C, the second step takes place and the morphinate splits into codeine and dimethylaniline as follows :

$$(7)\ M.ON(CH_3)_3C_6H_5 \longrightarrow M.OCH_3 + C_6H_5N(CH_3)_2$$

When anhydrous conditions were to be maintained throughout these reactions, commercial morphine base could not be used as such, because of its water of crystallization. It releases this water only slowly when heated to at least 110°C, and prolonged heating can cause partial decomposition of the alkaloid. The water is, however, released rather easily and without any damage to the alkaloid, when the latter is heated with toluene, which boils at 111°C and causes the water to distil off in an azeotropic mixture at a temperature somewhat below the boiling point of the toluene.

The methylation can be carried out as follows. The commercial morphine base is suspended in pure toluene by stirring. The mixture is heated to bring about distillation, which starts between 90°C and 100°C and is continued until the boiling point reaches 111°C. The remaining suspension mixture, which is now perfectly free of water, is kept protected from humidity and cooled down to 14°C under stirring. Then, an appropriate quantity of the titrated solution of the methylating agent is added, this quantity containing an excess of 5%. The morphine dissolves immediately, and the solution is then brought to boiling, which starts at about 80°C. The alcohol distils off with a part of the toluene and distillation is continued until the boiling point again reaches 111°C. The remaining solution contains now codeine and dimethylaniline, but does not contain any morphine. The codeine can be extracted in the usual manner with dilute mineral acid, whereby the aqueous phase is kept only slightly acid to litmus (pH 4 - 4.5) to prevent dimethylaniline from being extracted together with the codeine.

The reactions are quantitative, and no morphine remains unchanged, nor is morphine lost by decomposition or

secondary reactions, when temperature and moisture are carefully controlled. This method has been used successfully in commercial-scale manufacture for several years, and under favourable conditions the yield of codeine can reach up to 98%. It has been applied so far only to the methylation of morphine. Taking into account the ease with which the reactions proceed to completion under rather mild conditions, it can be expected that it will prove useful also for the methylation of phenolic hydroxyl groups in other nitrogen containing compounds.

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The quantitative assay of the principal alkaloids in the poppy and in opium by micro-measurement

By S. Pfeifer
Communication from the Pharmaceutical Institute of the Humboldt University, Berlin (Prof. Dr. Weiss)

Morphine being one of the most important alkaloids used in medicine, it is only natural that methods for the quantitative determination of morphine in opium, in the poppy and in galenical preparations should occupy an important place in the literature on alkaloid analysis. Descriptions of methods for the separation and determination of the by-alkaloids in the poppy and in opium, by contrast, are far less numerous. Moreover, these descriptions concentrate mainly on opium, and could not be applied as they stand, since far smaller amounts of these alkaloids occur in the poppy. Methods for the quantitative determination of the main alkaloids in opium or in total-alkaloid preparations have been described, *inter alia*, by E. Anneler (1), B. Kljatschkina (2), and D. C. Adamson, F. P. Handisyde & H. W. Hodgson (3). A spectrophotometric method for investigating Pantopon [proprietary name] has also been published by M. S. Dyer & A. J. McBay (4), but this is so far suitable only for pure-alkaloid mixtures. As far as we know, no method of measuring the codeine, thebaine, narceine, narcotine, papaverine, and narcotoline content, as well as the morphine content of the poppy plant, has yet been elaborated. The few authors to have reported at all on the by-alkaloid content have determined only the total by-alkaloid content, or have isolated these alkaloids from large quantities of drugs. Thus, W. Küssner (5), in his interesting experiments, used 10 g of non-phenolic alkaloids in each case and separated them by a series of analyses based on a modified form of Anneler's method. To obtain these 10 g of alkaloids, some 10 kg of drugs is required. A procedure of this kind is quite unsuitable for serial experiments — e.g., for following the alkaloid development in growing plants. There seemed, therefore, to be two good reasons for devising a method for the quantitative determination of the most important alkaloids in the poppy which entailed only slight expenditure of materials and required only a reasonable amount of time — namely, (1) the classification of types of poppy by alakloid groups, for cultivation purposes;[1] (2) determining the alkaloids during growth, in order to ascertain the biogenetic connexions of the poppy alkaloids.

In this connexion, it should be noted that, despite a number of works dealing with the qualitative or quantitative development of the alkaloids in the poppy plant, research into the biosynthesis of these alkaloids is still in its infancy. Moreover, research to date, which has dealt mainly with morphine and has often been determined by considerations of plant cultivation, has produced very conflicting results. Thus, according to A. Malin (8), the morphine content decreases as the poppy capsules ripen, whereas the codeine and narcotine contents allegedly increase. L. Fuchs (9) and, more recently, A. Guillaume & J. Faure (10) have made similar observations, the two last-named authors postulating that the oil and the morphine occur in inverse proportion to each other. A. Müller (11), reached the same conclusion with regard to the total alkaloid content. H. Baggesgaard-Rasmussen & O. Lanng (12) found that the morphine content varied with the climate and the time of the harvest. W. Küssner (loc. cit.), E. Wegner (13), and J. Tomko (14), however, found that the morphine content rose continuously until the capsules ripened. These studies are, however, again contradicted by a recent publication by M. Kučera (15), who found that the morphine content decreased as the plant ripened. W. Poethke & E. Arnold (16) who, in a model series of observations, followed the morphine content in the individual parts of the plant during growth, explained these differences by outside influences, particularly that of the weather; one year they found the highest morphine content in fully ripened capsules, the following year in the unripe capsules. Generally speaking, this is confirmed by our own research covering several years. J. C. Jespersen (17), I. v. Kabay (18), and H. M. Wüst & A. J. Frey (19) have also given data on the morphine content of leaves, head, and stems. According to E. Wegner (20), the morphine originates in the root. Opinions are also very much divided as regards the increase or decrease of alkaloids during storage. Our own observations suggest that no appreciable decrease in morphine content need be expected even after several years' storage, provided the drug be well dried. We observed no decrease whatever after storage for four years.

The few works containing data on the content or origin and distribution of the other alkaloids are predominantly qualitative studies. Some of the older publications must be viewed with caution, since the research in question was conducted, in part at least, with inadequate analytical aids. M. Kerbosch (21), who observed the formation of the alkaloids during growth with particular care, established the following order of importance : narcotine, codeine, morphine, papaverine, narceine, thebaine. Only narcotine and an " amorphous " alkaloid could be found in the seeds, which in part confirms and in part contradicts earlier findings (22-26). L. v. Itallie (27) summarized these data, together with the results of other studies conducted at the Pharmaceutical Institute at Leyden. Quantitative data on the formation of the by-alkaloids have been given by J. Tomko (loc. cit.), who, however, deals only with their total content. J. F. Reith, A. W. M. Indemans & W. R. Becker (28) have also supplied

[1] In Poland, for example, narcotine, codeine, and thebaine (6, 7) are obtained from ripe poppy heads as well as morphine; efforts will obviously be made to produce a variety exhibiting the highest possible content of by-alkaloids.

information regarding the total alkaloid content of ripe capsules and have compared various species. In an earlier work (29) these authors noted that the morphine content of the stem increased from the bottom to the top. According to C. Braga (30), there is neither decrease in the total alkaloid content nor displacement in the composition of the individual alkaloids when ripe poppy capsules age. K. Köver & V. Cieleszky (31) ascertained the narcotine content of various varieties of poppy by means of a polarographic method. Quantitative data regarding the individual by-alkaloids in the ripe capsule are also given by W. Küssner (loc. cit.). These, however, must also be regarded as incomplete, since no account is taken of narcotoline. Now, according to our investigations to date, the narcotoline content of ripe capsules in most cases exceeds the quantity of the other alkaloids several times over. We were also able to show with several varieties of poppy (32) that the narcotoline increases in quantity as the poppy ripens, and also in storage. G. Baumgarten (33) had already made similar observations regarding storage. These observations seem to show that the narcotoline, an appreciable quantity of which is present in the ripe capsule, originates from the partial demethylization of narcotine. This is also suggested by Kerbosch (loc. cit.), who ascertained by quantitative assay that large amounts of narcotine occur even in the young plant, and that this content increases with growth, but again decreases as the seed capsules ripen. Kerbosch found the highest content in the bud. H. Thoms (34) had already observed previously that before the plant blossomed there was appreciably more narcotine than morphine or codeine. Since according to Kerbosch codeine also occurs before morphine, it seems probable that in this case the o-demethyl alkaloids are the end-products of biosynthesis. This would admittedly conflict with the widely held view that the oxygen-methyl compounds are end-products, in which the methyl groups are fixed in such a way that they can no longer be mobilized by the organism (c.f. H. B. Schröter (35)).

The purpose of the procedures described below is to make it possible to supply quantitative findings on the formation of the by-alkaloids during growth and storage as well. The attempt to adapt the experiment for the investigation of opium also is prompted by the fact that the scientific investigation of the origin of opium plays an important part in the control of smuggling and illicit traffic (36). Conclusions regarding the origin of opium can be drawn from both quantitative and qualitative details of its alkaloid composition. In devising a method of this kind, special attention was paid to using only small quantities of drugs. In practice, this meant that only photometric or polarographic methods could be considered. As the alkaloids contained in an extract cannot be determined separately by photometric or polarographic means, a separation process had first to be devised; this we based partly on earlier studies (1-4).

The main difficulty was that, as against the classic methods of Anneler or Kljatschkina, precipitation reactions had to be discarded. The individual alkaloids could therefore be isolated only by extracting specimens of the drug with suitable solvents at various pH values. For this, it is essential that the individual alkaloids be completely separated in each case. Unlike extraction or precipitation processes in macro-analysis, where small quantities of residual alkaloids can generally be neglected, micro-analysis may be seriously affected by such tests. In the method given below, the quantitative determination of the individual alkaloids is discussed and a method given for separating them quantitatively. In experiments conducted along these lines, consistent results were obtained throughout, and were confirmed in various ways by comparative evaluation and checked by paper-chromatographic methods, as described below.

Quantitative Determination of Individual Alkaloids

Unfortunately, hope that Dyer & McBay's spectrophotometric process (loc. cit.), which enables alkaloids to be determined in the ultra-violet field, could be used for all alkaloids was not fulfilled, since there are still so many impurities present in the solutions of the individual alkaloids under investigation that any assessment, especially below a wavelength of 300 mμ, becomes impossible. Narcotine and papaverine solutions are alone sufficiently purified by the various separation processes for their absorption not to be appreciably disturbed at 312 mμ.

1. *Morphine*

Given our aim of determining minute quantities of alkaloids, some gravimetric methods for determining morphine described in the literature may here be ignored. Similarly, of the numerous colour reactions of morphine, only a few are of use for quantitative determination, notably the formation of 2-nitrosomorphine, the diazo-reaction and the iodic acid reaction. H. Wieland & P. Kappelmeier (37) found that morphine in acid solution mixed with nitrite gives a yellow tint, which shades into orange-red when aqueous ammonia is added. This nitroso reaction was first suggested for the quantitative determination of morphine by R. Fabinyi (38). It was subsequently elaborated by F. A. Goin (39) into a quantitative method of determining the morphine in opium. The accuracy of the method, according to C. G. van Arkel (40), could be improved by using a Pulfrich photometer. Sarrat (41), E. F. Heeger & K. H. Bauer (42), and, more recently, D. C. M. Adamson & F. P. Handisyde (43), W. Poethke & E. Arnold (44), J. F. Reith, A. W. M. Indemans. & W. R. Becker (29), R. L. Stephens (45), E. Wegner (46), B. Acacić, D. Marković & J. Petricić (47), A. Denoël (48), J. S. N. Cramer & J. G. Voermann (49), H. Baggesgaard-Rasmussen (50), A. B. Svendsen (51) and A. Bratina, M. Perpar & M. Tišler (52) also used this nitroso-morphine reaction for the quantitative determination of morphine, diacetylmorphine, and the morphine in opium and in parts of the poppy plant. The reaction has other uses too, since morphine can be made susceptible to polarographic determination — in opium and the poppy also — via the formation of 2-nitroso-morphine and its reduction (53-58). The diazo reaction is one often used for the quantitative colorimetric analysis of phenols. It is also the basis of the method of morphine assay proposed by L. Lautenschläger (59) and later, with modifications, by L. David (60) and by E. J. Ginsburg

TABLE 1

Narcotoline and morphine content of various types of poppy capsule

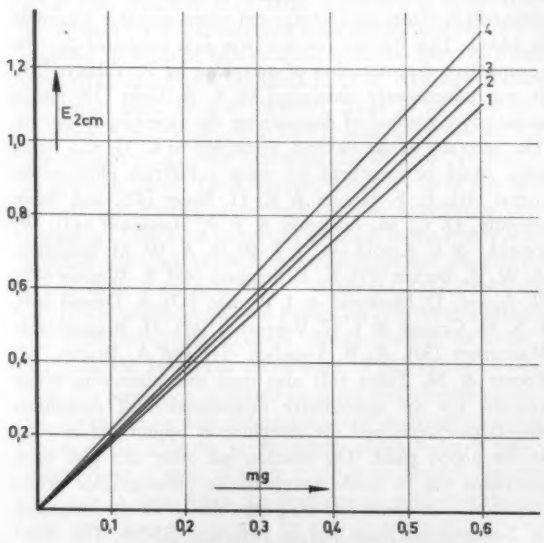
Sample	Morphine content	Narcotoline	Cotarnoline	Total narcotoline	Ratio of total narcotoline to morphine
I. Kleinwanzleben	0.23	0.022	< 0.001	0.022	9.5
II. Hybrid	0.36	0.017	< 0.001	0.017	4.8
III. Mahndorf	0.29	0.019	< 0.001	0.019	6.5
IV. Hybrid	0.40	0.01	< 0.001	0.01	2.5
V. Schlanstedt	0.26	0.093	0.012	0.105	40.5
VI. Tourneur Frères, type B[a]	0.68	0.048	0.014	0.062	9.1
VII. Blue poppy from Poland[b]	0.35	0.16	0.02	0.18	51.3
VIII. Poppy from the East[a]	0.37	0.197	0.013	0.21	56.7
IX. Native poppy 8[a]	0.61	0.136	0.024	0.16	26.6
X. Unknown	0.52	0.194	0.006	0.20	38.4
XI. Russia 2[a]	0.48	0.13	0.005	0.135	28.2
XII. Tourneur Frères, type D[a]	0.31	0.105	< 0.001	0.105	34.0
XIII. Unknown	0.51	0.154	0.016	0.17	33.3
XIV. Unknown	0.33	0.075	0.008	0.083	25.2
XV. Unknown	0.44	0.135	0.01	0.145	35.2

[a] Origin: Schlanstedt variety.

[b] Origin: Schlanstedt.

& N. J. Gawrilow (61). P. Balák & A. Jindra (62) also used this method recently. According to them, m-nitraniline and p-nitraniline are best suited for coupling. H. Auterhoff (63) investigated the quantitative ratio of the coupling elements and the optimum lye concentration for various substances, including morphine. The iodic acid reaction, first used for morphine determination by L. Georges & A. Gascard (64), was later successfully tried by A. Heiduschka & M. Faul (65) for the determination of morphine in poppy capsules. According to L. Szabolcz (66), solutions of morphine react with iodic acid by separating the iodine. When NaOH is added, the solution turns orange-yellow. This colour, caused by the hydroxyl of the morphine, also lends itself to colorimetric determination. This reaction has recently been refined by A. Mariani, S. Guarino & O. Marelli (67) to an exact spectrophotometric method for the determination of the morphine in opium. C. F. Moorhoff (68) adopted this method for assay in poppy capsules. J. N. S. Cramer & J. G. Voermann (69) and R. R. A. Pride & E. S. Stern (70) made further modifications by using nickel salts instead of iron (III) chloride. E. Brochmann-Hanssen (71) adopted the methods of the last-named authors in determining the morphine in opium after

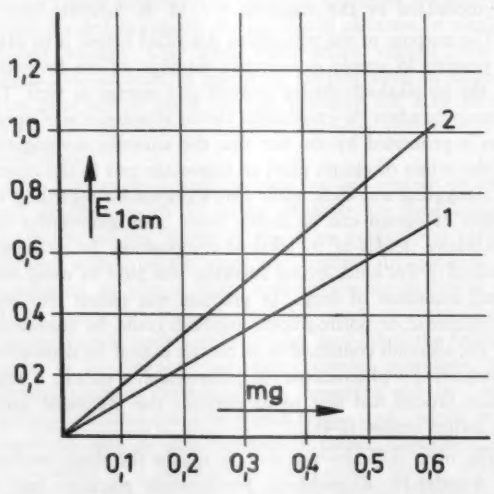
Figure 1



Effect of narcotoline on the nitrose reaction of morphine
(Method of *Wegner*, 2-cm vessel, 470 mμ)

1. Morphine
2. Morphine + 10% narcotoline
3. Morphine + 15% narcotoline
4. Morphine + 30% narcotoline

Figure 2

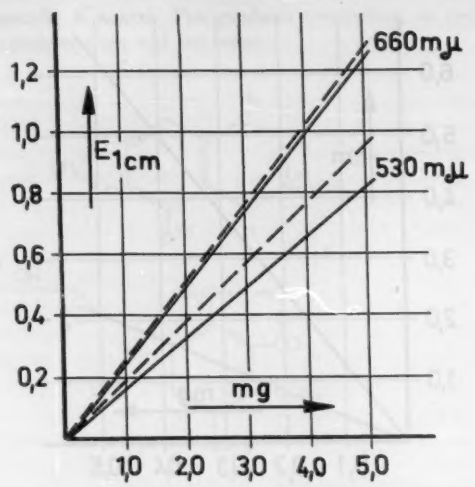


Effect of narcotoline on the diazo reaction of morphine
(470 mμ, 1-cm vessels)

1. Morphine
2. Morphine + 10% narcotoline

previously separating the morphine with ion exchange resins. These reactions are based exclusively on the presence of a phenolic hydroxyl group in the morphine molecule. It is thus not surprising that various other phenol alkaloids present in the poppy plant and other phenols should give similar reactions; in general, therefore, the morphine must be exactly separated before the determination itself is undertaken. The separation of non-alkaloid phenols presents no difficulties in this connexion. Of the other poppy alkaloids with a phenolic hydroxyl group (laudanine, laudanidine, codamine and narcotoline), narcotoline alone occurs in the poppy in any considerable quantity, sometimes as much as 30% to 50% of the morphine level (72, cf. table I). It was therefore necessary to discover whether narcotoline reacted with the reagents mentioned by colouring. This it did in all cases. Subsequent checks of morphine and narcotoline mixtures showed that the morphine values were in each case affected by narcotoline to a greater or lesser extent (73) (figs. 1-4). Only appreciable amounts of narcotoline were taken into account in each case. These studies, which were also used for determining the morphine content of the poppy head, clearly showed not only that the presence of narcotoline sometimes gives incorrect results for morphine, but also that in some cases the requisite conditions simply do not exist, as in the determination of morphine in poppy capsules by Pride & Stern's method (loc. cit.). These authors investigated the iodic acid test described by Cramer & Voermann (loc. cit.) to see whether it could not be adapted for the determination of morphine in opium and poppy capsules. They found that no poppy alkaloids give a colour reaction except pseudomorphine, which is sometimes detected in opium and in poppy capsules, and turns brown with iodic acid, and a deeper brown when an ammoniacal solution of nickel-chloride is added. In view of the difference in the extinction maxima (morphine; 670 mμ; pseudo-morphine; 530 mμ), the authors used a correction factor to evaluate the morphine value arrived at by computation and experiment, in order to eliminate error caused by pseudomorphine. In addition, the extinction was measured at both wavelengths. Narcotoline was disregarded. Our own experiments showed that narcotoline develops an olive-green colour [under experimental conditions], the intensity of which is overshadowed by the deep green of the morphine. Photometric measurements showed that narcotoline had practically no effect on the morphine value at 660 mμ, but did show an effect at 530 mμ (Fig. 4). The relevant absorption curves (fig. 5) help to explain this. To extract the morphine from the poppy capsule powder

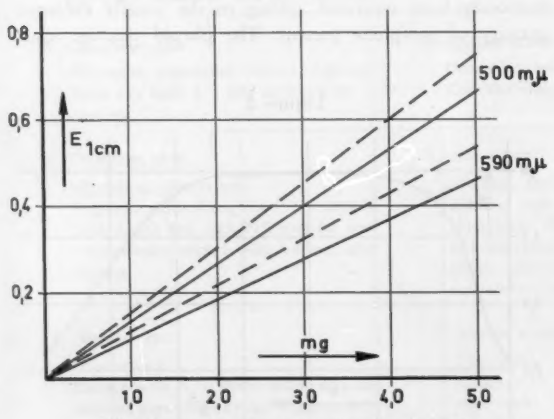
Figure 4



Effect of narcotoline on the iodic-acid reaction of morphine
Method of Pride & Stern (660 and 530 mμ, 1-cm vessel)

——— Morphine
— — — — Morphine + 30% narcotoline

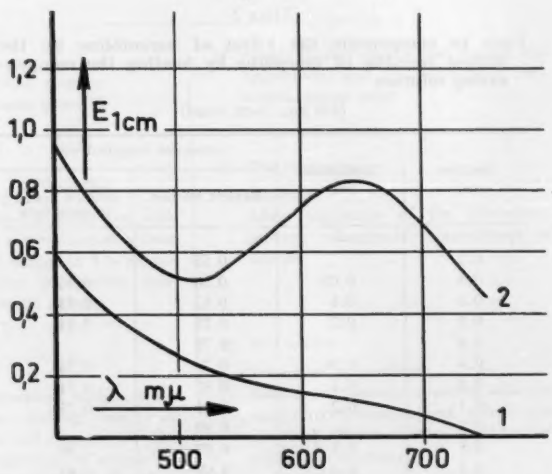
Figure 3



Effect of narcotoline on the iodic-acid reaction of morphine
Guarino's method (modified by *Moorhoff;* 500 and 590 mμ, 1-cm vessel)

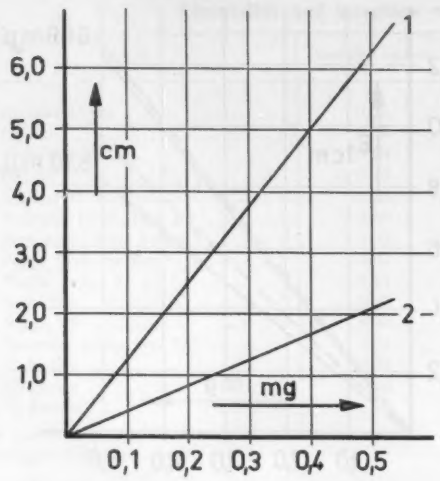
——— Morphine
— — — — Morphine + 30% narcotoline

Figure 5



Absorption curves for morphine and narcotoline (iodic-acid reaction)
(Method of Pride & Stern, using 3 mg of each alkaloid, 1-cm vessel)

Figure 6



Difference in height of vertical axis for various concentrations of morphine (1) *and narcotoline* (2)

according to Pride & Stern's method, the former is first alkalized with a sodium-carbonate solution and shaken with a benzene-n-butanol mixture (1 + 1) for one hour, after which the solvent is drawn off by suction and the residue washed. The morphine is separated from the solution with sulphuric acid and determined by spectrophotometric means in the sulphuric-acid solution without further purifying. The narcotoline thus passes almost completely into the sulphuric-acid solution. Although at 660 mμ (maximum absorption of morphine) it has no effect on the morphine level in the quantities in question, additional measurement at 530 mμ may give a false impression that pseudomorphine is present. The morphine figure is then " corrected " in error, and the value arrived at is a purely illusory one.

TABLE 2

Tests to compensate the effect of narcotoline on the nitrose reaction of morphine by heating the compensating solution

(470 mμ, 2-cm vessel)

Morphine (mg)	Narcotoline (mg)	Extinction compared with	
		Reagent solution	Compensating solution heated in water-bath
0.3		0.55	
0.3	0.05	0.58	0.56
0.3	0.1	0.62	0.55
0.3	0.2	0.75	0.54
0.4		0.75	
0.4	0.05	0.79	0.76
0.4	0.1	0.82	0.74
0.4	0.2	0.91	0.75
0.5		0.89	
0.5	0.1	0.97	0.90
0.5	0.2	1.07	0.89
0.5	0.3	1.17	0.92

Narcotoline is also included during polarographic determination of morphine (57), but any erroneous results usually fall well within the margin of error (fig. 6; table 3). The nitroso reaction seemed the most suitable for the methods discussed here, its reliability and high degree of sensitivity having been demonstrated in numerous studies. Disturbances due to narcotoline can, moreover, be largely eliminated in fairly simple fashion, without complicated separation, since it can be dissolved in a slightly alkaline solution, as described later. The resulting cotarnoline turns a standard solution yellow in much the same way as the narcotoline in the test solution is tinted by nitrite and ammonia. A type of compensation thus takes place, as may be seen from table 2.

Special instructions for the selective determination of morphine in quantities of 0.1 to 0.3 g of the drug for the examination of poppy heads, or parts of poppy heads, are given in the experimental part, section IV (cf. also 57 and 74).

TABLE 3

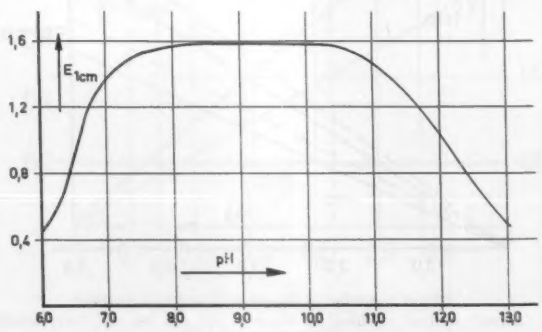
Effect of narcotoline on the polarographic measurement of morphine

mg alkaloid in 5 ml N/1 HCl		mg morphine found
Morphine	Narcotoline	
0.2	0.1	0.22
0.2	0.05	0.2
0.35	0.1	0.365
0.25	0.12	0.265
0.18	0.06	0.185
0.3	0.09	0.32

2. *Narcotoline*

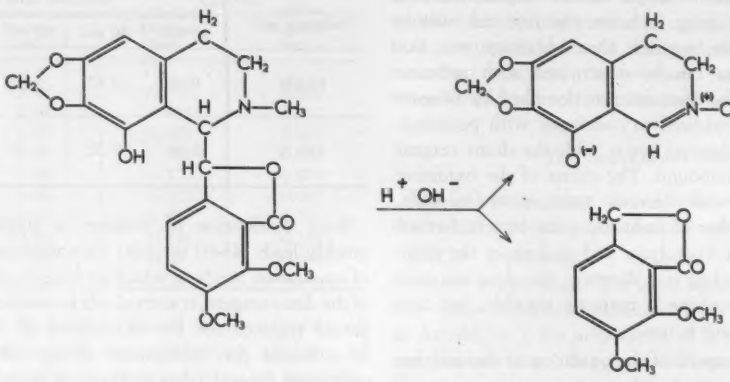
The phenol reactions to which reference has already been made (72) could also be evaluated quantitatively, but they are not suitable for determining the narcotoline unless it has previously been separated, owing to the usually excessive quantity of morphine present. The phenol reaction apart

Figure 7



Formation of Cotarnoline for various pH values of solution
(1 mg narcotoline, 420 mμ, 1-cm vessel)

from morphine can only be used after condensation with 4-amino-antipyrine in alkaline solution in the presence of an oxidizing agent (75). It is therefore worth noting that narcotoline can readily be separated in a slightly alkaline solution, especially if heated. This produces cotarnoline, an o-desomethylcotarnine, and meconine :



Ultraviolet and infra-red spectroscopic studies and polarographic examination showed that both cotarnoline and its salts are in fact quaternary ammonium compounds, while pH titration showed the base itself to be an intrinsic salt. By contrast, cotarnine has an aldehyde structure, and assumes a quaternary ammonium pattern only in the salt form. It was found that the formation of cotarnoline from narcotoline occurs quantitatively at pH = 8-10 (fig. 7). At pH values > 10 or < 8, conversion is not complete. The cotarnoline thus produced is bright yellow, and enables the narcotoline to be determined quantitatively from 0.05 mg upwards. Although narcotoline has recently been found in opium too (78), its determination in opium was disregarded owing to the low content (< 0.05%).

Method of separating the Principal Poppy Alkaloids

1. Extraction of alkaloids from opium with N/2 sulphuric acid; extraction from the poppy plant after alkalization of same with sodium-carbonate solution together with a mixture of methylene chloride, ether, and methanol in the ratio of 60 : 30 : 10. Following evaporation of the solvent mixture in a vacuum, the residue is absorbed with ether and shaken out with N/2 sulphuric acid. The sulphuric-acid solution is separated and (for poppy extracts only) mixed with trichloro-acetic acid; an aliquot portion is used for measuring the *narcotoline*; another is adjusted with aqueous ammonia to a pH of 7-8 and extracted with chloroform.

2. *Chloroform phase*
(Narcotine, papaverine, codeine, thebaine, narceine, morphine, and narcotoline in part only). Shake out with N/10 soda-lye.

3. *Aqueous phase*
(Morphine, narcotoline, narceine)

4. *Chloroform phase*
(Narcotine, papaverine, codeine, thebaine)
Shake out with 1 : 500 tartaric-acid solution.

5. *Alkaline extract*
(Morphine, narcotoline, narceine)
Combine with aqueous phase (3).

10. Acidify alkaline solution with concentrated hydrochloric acid. Perforation with chloroform (opium only).

6. *Chloroform phase*
(Narcotine, papaverine)
Evaporate chloroform, dissolve residue in alcohol, and heat with N/1 soda-lye over a water-bath; extract with benzene after cooling.

7. *Acid extract*
(Codeine, thebaine)
Alkalize with soda-lye, shake out with chloroform. After compression of solvent and absorption of residue in 4 N/10 sulphuric acid, perform photometric measurement of *codeine* and *thebaine*.

11. *Chloroform phase*
(Narceine)
After evaporation of the chloroform, perform polarographic measurement of *narceine*.

8. *Benzene extract*
(Papaverine)
Extract with N/10 hydrochloric acid and measure papaverine by spectrophotometry at 312 mμ.

9. *Alkaline solution*
(Narcotine)
Acidify with hydrochloric acid, heat in a water-bath; after cooling, mix with aqueous ammonia and extract with chloroform. After evaporation of solvent and absorption of residue in N/10 hydrochloric acid, measure *narcotine* by spectrophotometry at 312 mμ.

12. *Acid solution*
(Morphine, narcotoline)
Alkalize with aqueous ammonia, shake out with chloroformisopropanol. After evaporation of solvent mixture, measure *morphine* by photometry.

3. *Codeine and Thebaine*

Quantitative *photometric* investigation of the thebaine and codeine content of poppy capsules has not yet been undertaken as far as we know. Since no simple method originally existed for separating codeine from thebaine, the first task was to assay the two alkaloids together. One advantage was that both alkaloids together can be determined with sufficient accuracy by means of the diazo reaction described by Wegner (79). Codeine can be oxidized to codeinone with potassium permanganate, and codeinone reacts with the diazo reagent to form a coloured compound. The excess of the oxidizing agent is eliminated with ferrous ammonium sulphate. Thebaine, as an enol ether of codeinone, can be transformed into the latter by acid hydrolysis, and undergoes the same colour reaction. According to Wegner, the determination of the thebaine with codeine is perfectly possible, but that of codeine with thebaine is not.

It was found that the speed of the oxidation of the codeine depends less on the time it takes the potassium permanganate to act than on the acidity of the solution. Wegner had already shown that the maximum extinction is reached after only 15 seconds. According to Wegner, a longer period (of oxidation) apparently leads to the formation of further oxidation products, which are likewise liable to undergo colouration in the presence of the diazo reagent. This is suggested, for example, by the differing tints (codeine, raspberry; thebaine, violet) and by the greater intensity in the case of thebaine. Unfortunately, it is not clear from Wegner's work what was the pH value of the codeine solution used for plotting the calibration curve. In the light of our findings, it must have been a very slightly acid solution; according to Wegner's rule, such a solution does in fact yield the highest extinctions. As the acidity of the solution is increased, oxidization of the codeine via codeinone to further oxidization products occurs appreciably faster (table 4); this is shown by the fact that

TABLE 4

Effect of acidity on oxidizing effect of potassium permanganate on codeine

Method of E. Wegner; 500 mμ, 1-cm vessel

Codeine (mg)	Acidity of solution (Sulphuric acid)	Extinction
0.2	0.01 N	0.68
	0.05 N	0.65
	0.1 N	0.62
	0.2 N	0.53
	0.3 N	0.46
	0.4 N	0.35
	0.5 N	0.32

the longer the oxidization agent takes to act, the smaller the extinctions become, being practically nil after as little as one minute (table 5). On the other hand, it was also found that, under identical experimental conditions (length of oxidization = 15 seconds) solutions prepared with sulphuric acid yield extinctions considerably lower than those suggested by Wegner's curve, yet consistent with Lambert-Beer's law.

TABLE 5

Oxidation of codeine in relation to time factor

Method of E. Wegner, 0.2 mg codeine; 500 mμ, 1-cm vessel

Acidity (Sulphuric acid)	Extinction after oxidation				
	15 sec.	30 sec.	45 sec.	60 sec.	120 sec.
N/100	0.68	0.67	0.65	0.65	0.61
4 N/10	0.35	0.22	0.07	0.03	0.01

Since oxidization of codeine in sulphuric-acid solution quickly leads (45-60 seconds) via codeinone to the formation of oxidization products which no longer colour in the presence of the diazo reagent, it seemed safe to assume that the 15-second period required for the oxidization of codeine might also be sufficient for oxidization of the relevant amounts of codeinone formed from thebaine to produce colourless solutions with the diazo reagent. This assumption proved correct when thebaine was hydrolized for 20 minutes and then treated in the same way as codeine (table 6). It is thus possible to

TABLE 6

Effect of acidity on oxidizing effect of potassium permanganate on codeinone (from thebaine), using Wegner's method for codeine

(500 mμ, 1-cm vessel)

Thebaine (mg)	Acidity of solution (Sulphuric acid)	Extinction
0.2	N/100	0.35
	N/20	0.32
	N/10	0.28
	N/5	0.16
	3N/10	0.09
	4N/10	0.03
	N/2	0.02
0.15	4N/10	0.01
0.10	4N/10	0.02
0.05	4N/10	—

determine both alkaloids together. For determining the codeine one needs only to heat the sulphuric-acid solution containing both alkaloids before oxidization for some time in a boiling water-bath. The optimum acid concentration, on the basis of the findings given in tables 4 and 5, is 4N/10. With this degree of acidity, the codeinone already present at the beginning of oxidization is oxidized fast enough and the extinctions, even for minute quantities of codeine (0.04-0.2 mg) are still large enough to permit of sufficiently accurate measurement, as shown by table 7. The photometric determination of thebaine is somewhat less accurate than that of the other alkaloids (approximately $\pm$ 10 per cent). The reason for this is apparently that with hydrolysis not only codeinone, but also other products appear, as analysis of the hydrolosis products by paper chromatography showed. With increasing hydrolosis, for example, a second diazopositive compound occurs. To ensure sufficient accuracy, then, the time prescribed

TABLE 7

Documentary analyses for the determination of codeine and thebaine together

Given		Observed	
Codeine (mg)	Thebaine (mg)	Codeine (mg)	Thebaine (mg)
0.04	0.08	0.041	0.078
0.06	0.04	0.058	0.037
0.08	0.08	0.083	0.082
0.1	0.02	0.095	0.018
0.12	0.08	0.12	0.074
0.14	0.05	0.135	0.049
0.16	0.10	0.17	0.105
0.18	0.20	0.185	0.185
0.20	0.08	0.20	0.085

for hydrolosis must be exactly adhered to. Investigation of the hydrolosis products and the oxydization products of codeine under the conditions described has not yet been completed.

4. *Narcotine and Papaverine*

Certain methods for the quantitative photometric and polarographic assay of these alkaloids already exist (31, 79, 80, 81). Of these, only the polarographic method for determining the narcotine content of the poppy has apparently found practical application so far. The methods described by J. Holubek & J. Volke (79) and by K. Köver & V. Cieleszky (loc. cit.) are based on splitting the narcotine by oxidation into cotarnine and opianic acid, which can be reduced by polarography.

For the photometric determination of the alkaloids referred to, E. Wegner has given a fluoroscopic photometric method for the determination of papaverine (81) and a method based on the oxidation of narcotine (79) with subsequent treatment of the reaction products with diazobenzene sulphonic acid. Since neither method proved entirely satisfactory in our investigations — least of all as applied to drug extracts — the ultraviolet spectrophotometric method seemed the most suitable. Both alkaloids have a maximum at 312 mμ. The method therefore presupposes the separation of the narcotine from the papaverine, which is easily done by splitting the lactone of the narcotine.

5. *Narceine*

No attempts have hitherto been made to determine the narceine contained in the poppy, the quantities observed in various paper-chromatographic investigations having been extremely small. The narceine content of opium must be determined polarographically, since the colour reactions described in the literature (82, 83) are so insensitive that the test sample would have to be about ten times as large before any useful results were obtained. In acid conductivity solutions, narceine effects a well-defined step in the bi-electronic reduction of the keto group conjugated with the double bond. Its half-stage potential is independent of the concentration of the alkaloid, but varies with the pH of the solution (84). In this connexion, it was important to note that meconic acid is also directly reducible by polarography (85). Furthermore, the half-stage potentials of these two compounds differ by only 0.2 volts, a fact which seriously interferes with the determination of the narceine through meconic acid — which is in any case present in opium in excessive amounts. It can, however, be shown that, under the conditions used for extracting narceine, the solution used for polarographic measurement is free from meconic acid.

THE SEPARATION PROCESS

The following points should be noted with regard to the method of separating the alkaloids given below.

1. The application of heat is avoided in extraction from the poppy plant. The usual methods of soxhlet extraction using methylene chloride (Küssner, Wegner, Poethke & Arnold, etc.), the preparation of hot extracts with benzene and alcohols (e.g., Baggesgaard-Rasmussen & Lanng), and the preparation of aqueous-acid extracts are all unsuitable, partly owing to the sensitivity of narcotolin (72) in slightly alkaline solution to heat, and partly owing to the formation of emulsions during subsequent isolation of the alkaloids.

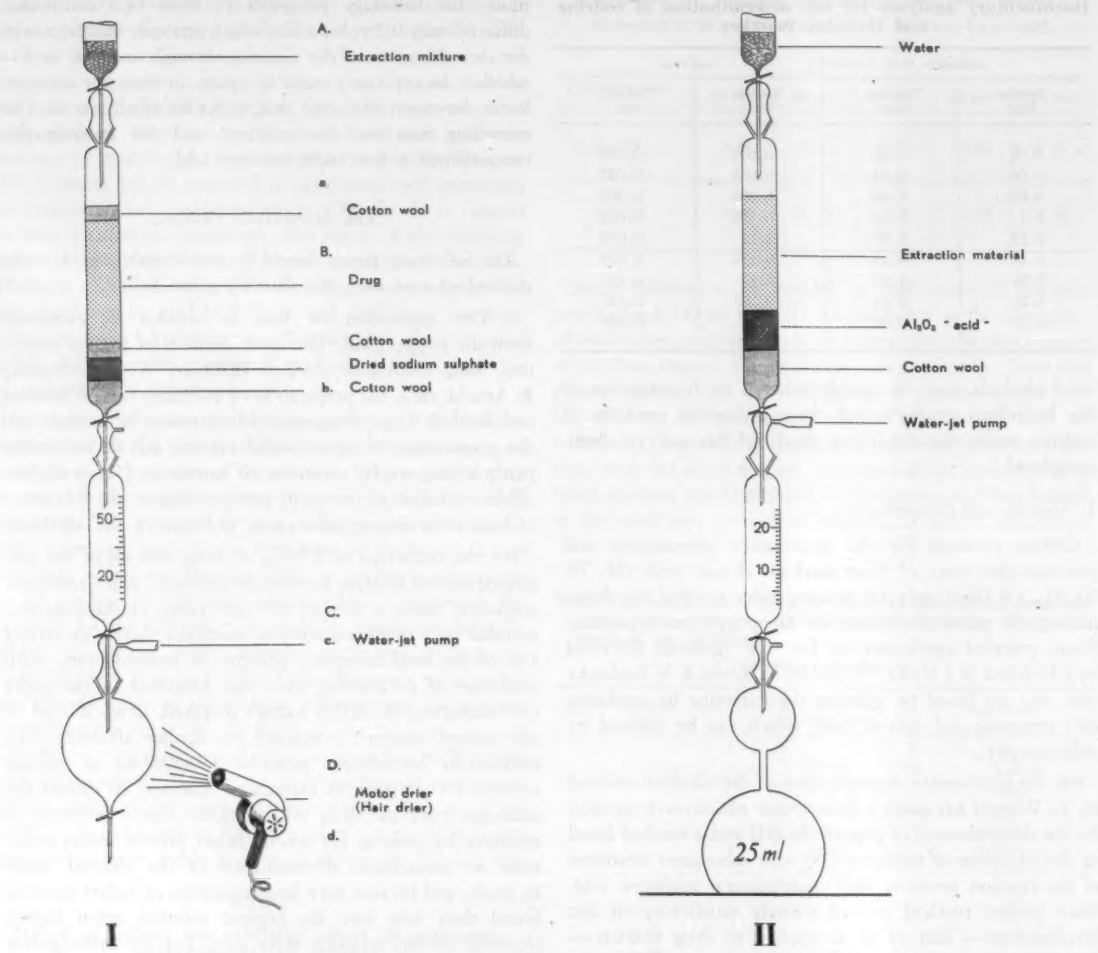
For the extraction of 2.5-5 g of drug, 100 ml of the prescribed solvent mixture is normally sufficient. After additional extraction using a further 100 ml, paper chromatography revealed only small quantities of morphine, barely exceeding 1% of the total morphine content. In isolated cases, small quantities of cotarnoline were also identified in the paper chromatogram. A further extract prepared, using 100 ml of the solvent mixture, contained no further alkaloids. The instructions accordingly prescribe 150-200 ml of solvent mixture for purposes of extraction. Attempts to extract the alkaloids from the drug with various organic solvents or mixtures by shaking for several hours proved unsuccessful, since no quantitative determination of the alkaloid could be made, and because very large quantities of ballast material found their way into the organic solution when highly alcoholic solvent mixtures were used. For the investigation of opium a sulphuric acid extract is used.

2. To save time, compression of the solution containing the alkaloid takes place in a vacuum at normal temperature during the actual extraction process (fig. 8, I).

3. The addition of trichloracetic acid to the acid-extract liquid helps to eliminate ballast materials and largely prevents emulsification during subsequent isolation of the individual alkaloids. Extraction with trichloracetic acid instead of sulphuric acid is inadmissible, since parts of the narcotine, papaverine and narcotoline may remain in the ether. When investigating opium, trichloracetic acid is not added, since it interferes with the polarographic determination of the narceine.

4. In order to simplify the method further, an attempt was made to extract narcotine, papaverine, thebaine, and codeine from the acid-extract solution after alkalization with soda-lye. However, paper chromatography showed that the alkaline liquid still contains narcotine and papaverine, presumably owing to the presence of dissolving agents. These

Figure 8



alkaloids were therefore first converted to the organic phase at pH 7.5-8.0 by means of chloroform. In this way, narcotine and papaverine can be determined quantitatively, as well as codeine and thebaine. However, since morphine, narceine, and narcotoline are also partly extracted at this pH, the latter have subsequently to be separated with soda-lye; during this process, the narcotine and papaverine are completely retained in the chloroform.

5. The narcotine and papaverine are separated from the codeine and thebaine by extracting the chloroform solution with a 0.2% tartaric-acid solution. Experiments with more concentrated solutions of tartaric acid showed that papaverine in particular passes partly into the acid phase. Conversely, attempts to separate the codeine and thebaine by using acid buffer solutions produced incomplete separation.

6. To separate narcotine from papaverine, the narcotine, in alkaline solution, is split with lactone. The papaverine can be extracted with benzene from the alkaline liquid, the narcotine of which is henceforth hydrosoluble.

7. The quantitative extraction of narceine proved extremely difficult. Neither frequent shaking out of hydrochloric-acid solutions with chloroform nor extraction with the same solvent at the iso-electric point met with success. The only method whereby narceine could be quantitatively converted from hydrochloric-acid solution into chloroform was by perforation, using a Schott perforator.

8. The alkaloids laudanine and laudanidine also had to be specially considered when investigating opium. Although they are phenol bases, they occur largely in the codeine-thebaine fraction, and are thus extractable with chloroform from sodium oxide alkaline solution, as previous reports have shown (86). This is of some consequence, since both alkalis colour in response to the diazo reagent. Despite the lesser colour intensity of these reaction products by comparison with that of codeine and thebaine, the danger of error could not be entirely excluded, particularly since paper chromatography of various kinds of opium showed the content to be not inconsiderable. When a sulphuric-acid solution, obtained

in the course of analysis from commercial opium and containing laudanine and laudanidine as well as codeine and thebaine, was mixed with a diazo reagent and an alkaline lye, an orange tint with measurable absorptions, which could not originate either from thebaine or from codeine, did in fact appear. However, it was found that the orange tinting of the alkaloids with the diazo reagent absorbed practically no light at the wavelength of 570 mμ suited to the measurement of thebaine (the absorption maximum for laudanine and laudanidine is about 470 mμ), and that in the course of the operations required for performing the colour reaction for codeine (heating in sulphuric-acid solution, oxidation with potassium permanganate) laudanine and laudanidine also undergo changes and the resultant reaction products no longer give a diazo reaction.

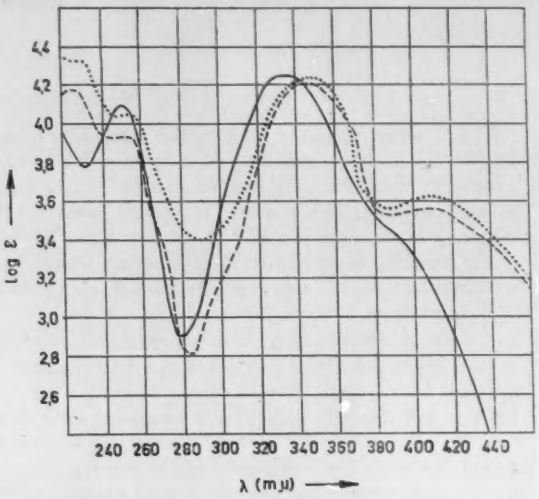
9. With the narcotoline content, it is also possible to determine the content of cotarnoline (72, 76, 77). Generally speaking, however, the figure was included with that for narcotoline, since cotarnoline is not really a genuine alkaloid in its own right.

The separation process described was, as already indicated, checked for accuracy by paper chromatography, in accordance with a previously described method (87).

This was followed by the separation of the narcotine, papaverine, and narcotoline alkaloids, after splitting up the last-named into its cotarnoline component with ether saturated with aqueous ammonia. According to A. Bettschart (88), the separation of narcotine and papaverine can also be successfully performed on buffered paper (pH = 3.8) with water-saturated ether. Under these conditions, narcotoline can also be separated without previously decomposing the narcotine and papaverine (89). We accordingly adopted these variants in later experiments. Apart from traces of alkaloids not under discussion here, only the alkaloids expected were found in the individual fractions. The presence in the thebaine-codeine solution of laudanine and laudanidine, the separation of which by paper chromatography has not yet succeeded, has already been noted. Slight quantities of morphine which fell within the margin of error of the photometric method were found from time to time in the tartaric-acid solution of thebaine and codeine. In some cases this error can be eliminated by extracting the alkaline solution disintegrated with ammonium chloride and from which thebaine and codeine have previously been extracted with chloroform by means of a mixture of chloroform and isopropanol. This extract is added to the chloroform-isopropanol solution, which contains the largest quantity of morphine. The methods of photometric determination were also re-examined. The polarographic method of determining narcotine recently published by J. Holubek & J. Volke (70), for example, was found useful. Wegner's (loc. cit.) colour reactions for narcotine and his (loc. cit.) fluoro-photometric method for determining papaverine were also employed.

As already stated, neither method proved completely satisfactory. We were unable, for example, to reproduce the calibration curve for narcotine given by Wegner, obtaining instead a considerably flatter curve. Before being adopted by research laboratories not equipped with a universal spectrophotometer, these methods would require considerable revision and adaptation.

Figure 9



Ultraviolet spectra of cotarnoline in solutions of various pH values (——— pH = 2.5/ – – – – – pH = 7.2/ ..... pH = 12.2)

That the solutions employed for the spectrophotometric determination of narcotine and papaverine were sufficiently pure was proved by measuring the extinctions at various other wavelengths and by comparing them with pure solutions of the alkaloids of similar concentration. The values obtained for narcotoline were easily checked. The cotarnoline which occurs on decomposition of narcotoline, besides the maximum used for determination at 410 mμ also has maxima between 338 and 348 mμ (fig. 9) with considerably larger extinctions, both in acid and in alkaline solution. We attached special importance to comparative measurements at these wavelengths, since at 410 mμ only the yellow tint is measured; results might thus be affected by contamination caused by extractives. Finally, polarographic investigations (cotarnoline) can be reduced by polarograph (77) and also closely agree with photometric findings. The findings for morphine were also checked by polarograph (57). For codeine and thebaine, in particular, for which no comparative checks were possible and all other alkaloids, too, paper chromatography also demonstrated the correctness, by and large, of the values found. Lastly, some of the alkaloids were also determined by a recently developed method for quantitative paper chromatography by photographic means (90),

It should be added that, in the investigation of leaves or other parts of the poppy plant with a very low alkaloid content disturbances may be caused by emulsion formation owing to the high chlorophyll content or because of the larger weighed portion of drug sometimes necessary in certain cases. In such cases, it may be necessary to separate the aqueous from the organic phase by means of centrifugation.

Experimental part

I. Isolation and Separation of Alkaloids

1. *Analysis of Parts of the Poppy*

2.5 g of poppy capsules or 5.0 g of other parts of the poppy (in each case finely ground) are well saturated with 2 or 4 ml respectively of sodium carbonate solution (7.5 g anhydrous sodium carbonate, 92.5 g water) and left to stand for 30 minutes pressed against the sides of the mortar. At the end of this time, the moist powder is collected and poured into extraction tube B on to approximately 5 cm of dried sodium sulphate, as shown in fig. 8, I. From the reserve container A, a mixture of 60 parts by volume of methylene chloride, 30 parts ether, and 10 parts methanol is fed through tap b until the extract begins to drip into collecting vessel C. Tap b is then closed, to ensure better saturation of the drug with the solvent. At the end of 15 minutes, tab b is again opened and extraction continued without interruption, the flow through tap b being adjusted to approximately 80 to 100 drops per minute. The solution collected in container C is allowed to pass from time to time via tap c into the separating funnel D; the solvent in D is then brought to evaporation by suction with a water-jet pump. To avoid excessive icing of the separating funnel, it is advisable to apply a mechanical drier (e.g., a hair-drier) from the side. Extraction takes about 1½ to 2 hours. The residue is absorbed with 10 to 15 ml ether and is twice shaken out for 2 minutes with 10 ml of N/2 sulphuric acid. The acid extracts are collected in an Erlenmeyer flask and mixed with 1.0 g trichloro-acetic acid; the whole is well shaken, allowed to stand for a few minutes, and passed through a dry circular filter of 8 cm diameter into a 25-ml measuring flask. The Erlenmeyer flask and filter are twice rinsed with 2 ml of water and the contents of the measuring flask then diluted with water up to 25 ml (= solution A). 10 ml of this solution are used for further determination of narcotoline; the remaining 15 ml are rinsed with a little water, passed into a separating funnel, and adjusted to pH 7.5 to 8.0 by adding drops of 10% aqueous ammonia. The pH value of the solution is checked by means of a suitable indicator paper. When the solution passess from acid to alkaline values, its colour darkens, thus greatly facilitating adjustment of the pH. The solution, now slightly alkaline, is next shaken three times with 20 ml of chloroform, each time for 2 minutes, after which the combined chloroform solutions are extracted with 10 and 5 ml of N/10 soda-lye.[1] The alkaline solutions are added to the extracted fluid adjusted to pH 7.5 to 8.0 (= solution B), this solution is mixed with 2 ml of hydrochloric acid DAB.6 (DAB = German Dispensatory), poured into a 50-ml measuring flask, and mixed with 5 ml of 10% aqueous ammonia. The flask is filled up to the mark, after which the morphine is extracted from 10 ml (more in some cases) of this liquid with 20 and 2 × 10 ml of chloroform-isopropanol (3 + 1 parts by volume). The combined organic solutions are then compressed in the water-bath, the residue is absorbed with 5 ml of chloroform, and the latter shaken out with 20 ml of N/10 hydrochloric acid (= solution C).

The codeine and thebaine are separated from the chloroform solution extracted with soda-lye by shaking them with 10 and 2 × 5 ml of 0.2% tartaric-acid solution. The combined tartaric acid solutions are mixed with 1 ml of 15% soda-lye and twice extracted with 20 ml of chloroform. The organic solvent is evaporated over the water-bath and the residue absorbed with 10 ml of 4N/10 sulphuric acid, the whole being slightly warmed (= solution D). The remaining chloroform solution, containing narcotine and papaverine, is compressed, the residue mixed with 2 ml of alcohol and 5 ml of N/1 soda lye, and heated for 5 minutes in a boiling water-bath. After cooling, the mixture is extracted three times with 5 ml of benzene; the benzene solutions are then shaken with 5 ml of N/10 soda-lye once more, and the alkaline solution of the liquid containing lactone-separated narcotine is added. To isolate the papaverine, the benzene solution is extracted with 10 ml of N/10 hydrochloric acid (= solution E). To regenerate the narcotine, the solution containing it is mixed with 2 ml of 25% hydrochloric acid and heated for 30 minutes in a boiling water-bath. After cooling, aqueous ammonia is added until an alkaline reaction sets in and extraction performed twice with 20 ml chloroform; the solvent is

[1] Sometimes the soda-lye is not cleanly separated after the second extraction. In such cases, the main portion of the chloroform should be removed, a few ml of ether added, and the whole shaken once more. This ensures complete separation. The soda-lye is then removed and the ether-chloroform mixture added to the chloroform previously removed.

Table 8

Documentary analyses

Alkaloid	mg		mg		Poppy capsules %				Opium %			
	Given	Observed	Given	Observed	Observed	Added	Total	Observed total	Observed	Added	Total	Observed total
Morphine	4.0	3.9	3.0	3.1	0.58	0.2	0.78	0.76	10.6	3.0	13.6	13.9
Codeine	0.2	0.21	0.5	0.46	0.027	0.02	0.047	0.044	0.82	0.5	1.32	1.27
Thebaine	0.15	0.17	0.20	0.19	0.008	0.02	0.028	0.030	0.54	0.5	1.04	0.98
Narceine	0.2	0.18	0.10	0.11	—	—	—	—	0.24	0.2	0.44	0.47
Papaverine	0.5	0.48	1.0	1.05	0.015	0.10	0.115	0.107	1.2	1.0	2.2	2.08
Narcotine	0.5	0.50	2.0	1.95	0.047	0.10	0.147	0.140	3.3	3.0	6.3	6.5
Narcotoline	1.0	0.94	0.5	0.53	0.125	0.05	0.175	0.170	—	—	—	—
Cotarnoline (included as narcotoline)	—	—	—	—	0.030	—	0.030	0.030	—	—	—	—

then compressed over the water-bath and the residue absorbed with 10 ml of N/10 hydrochloric acid. After filtration, this solution (= solution F) is used for the spectrophotometric determination of narcotine.

2. *Analysis of Opium*

0.1 g of opium is accurately weighed, carefully crushed in a mortar with a few drops of N/2 sulphuric acid, and further diluted up to a total of 10 ml. The solution is poured into a measuring flask of 25 ml capacity and the mortar rinsed with 2 × 5 ml of N/2 sulphuric acid. The combined solutions are then diluted with water up to 25 ml. 15 ml of this filtrate are used for the separation processes described above. The narcotoline is determined at this stage. For morphine and narceine, special procedures apply (cf. II, 1b and II, 6).

For measuring the extinctions, when analysing opium, it may be necessary to use thinner vessels than those described. If necessary, the solutions must also be diluted (narcotine, codeine).

II. Determination of Individual Alkaloids

1. *Morphine*

(a) *Determination in Parts of the Poppy*

5 ml of filtered solution C are poured into a test-tube with a ground stopper, mixed with 2 ml of a 1% sodium-nitrite solution, and immediately shaken for 15 seconds. Exactly 15 minutes after the addition of the nitrite, the combination is mixed with 3 ml of 10% aqueous ammonia, after careful rotation with 2.5 ml of water. Five minutes later, with the aid of a suitable photometer,[2] the colour intensity is measured at 470 mμ (2-cm vessel) against a standard solution[3] prepared in the following way :

5 ml of solution C mixed with 3 ml of 10% aqueous ammonia and 2.5 ml of water, heated for five minutes in a boiling water-bath, and subsequently cooled.

[2] For all analyses, the universal spectral photometer of VEB Carl Zeiss, Jena, was used.

[3] This solution serves to compensate for any narcotoline that may be present (39).

(b) *Determination in Opium*

The acid liquid remaining in the Schott perforator after separation of the narceine is carefully poured, together with the chloroform also remaining there, into a separating funnel; the chloroform is separated and the acid solution poured into a measuring flask of 100 ml capacity. The perforator and the separating funnel are well rinsed with small amounts of water. 10 ml of 10% aqueous ammonia are added, and water is added to the solution in the flask up to the mark. The morphine is extracted from 20 ml of this solution with 20 and 2 × 10 ml of chloroform-isopropanol (3 + 1 parts by volume), the combined organic solutions are compressed in the water-bath, the residue absorbed with 5 ml of chloroform, and the latter shaken with 20 ml of N/10 sulphuric acid. After filtration, 5 ml of this solution are used for the determination of morphine, as explained under II, 1a above.

2. *Codeine*

3 ml of solution D are heated in a boiling water-bath for 20 minutes in a test-tube in which a smaller tube filled with water is suspended to avoid evaporation of water. After cooling, the solution is mixed with 0.5 ml of a 0.3% solution of potassium permanganate; the whole is immediately shaken, and oxidation is interrupted after exactly 15 seconds by adding 0.5 ml of a freshly prepared 10% solution of ammonium ferrosulphate. After 5 minutes, 2 ml of 15% soda-lye are added, after which the mixture is allowed to stand for one hour. It is then separated by filtration from the sediment, and 3 ml of the filtrate are mixed with 5 ml of a mixture of equal parts of diazo reagent I and II (DAB 6). The intensity of the ensuing raspberry colour is immediately measured against a similarly treated standard preparation at 500 mμ (2-cm vessel).

3. *Thebaine*

3 ml of solution D are heated for 20 minutes, as for codeine, and the resultant product mixed after cooling in cold water with 2 ml of 15% soda-lye and subsequently with 3 ml of a mixture of equal parts of diazo reagent I and II (DAB 6). The intensity of the ensuing violet shade is measured at 570 mμ (1-cm vessel).

Table 9

Results of analysis of opium and ripe capsules of various types of poppy

(Percentages)

Type	Morphine	Codeine	Thebaine	Narceine	Papaverine	Narcotine	Narcotoline	Cotarnoline (calculated as narcotoline)
Poppy I	0.41	0.027	—	—	0.008	0.06	0.075	0.015
Poppy II	0.58	0.027	0.008	—	0.015	0.047	0.125	0.030
Poppy III	0.67	0.032	0.01	—	0.018	0.022	0.050	0.024
Poppy IV	0.71	0.043	0.015	—	0.04	0.035	0.11	0.035
Poppy V	0.53	0.035	—	—	0.009	0.050	0.09	0.01
Opium I	10.6	0.82	0.54	0.24	1.2	3.3	—	—
Opium II	11.7	1.20	0.95	0.32	0.8	5.3	—	—
Opium III	9.9	0.7	0.42	Less than 0.1	1.2	2.8	—	—

4. *Narcotine, papaverine*

The tinctures of solutions E and F are measured at 312 mμ (1- or 2-cm vessel). (1- and 2-cm vessels respectively).

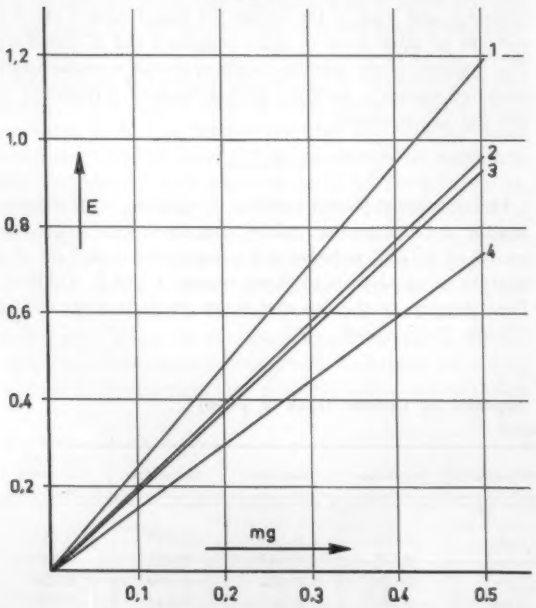
5. *Narcotoline*

10 ml of solution A are brought with aqueous ammonia as near as possible to a pH = 7 and subsequently extracted with 20 and 2 × 10 ml of a mixture of 4 parts by vol. of chloroform and 1 part amyl alcohol. The organic solution is then shaken with 10 ml of N/2 sulphuric acid, and 4 ml of the acid solution are mixed with 1 ml of 10% aqueous ammonia; a further 4 ml are mixed with 1 ml of water (= standard solution) and the intensity of the yellow shade produced in the ammoniacal solution is measured at 410 mμ (1-cm vessel).[4] By this means one can determine the amount of cotarnoline present in the drug; this is best included with the narcotoline (cf. also under 9). After measurement, the ammoniacal solution is poured into a test-tube and heated for 5 minutes in a boiling water-bath. It is then cooled and the tincture is measured once more as before. This represents the amount of narcotoline, including cotarnoline, present in the drug.

6. *Narceine*

To determine the narceine content of opium, solution B (cf I, 1) is mixed with 3 ml of concentrated hydrochloric acid and perforated with chloroform in a Schott perforator for about two hours. The solvent is then distilled, 5 ml benzene and 3 ml of N/10 soda-lye are added to the residue, the flask is closed, and shaken hard. 2 ml of 30% acetic acid are next added and the mixture is poured into a separating funnel. The acid component is separated, filtered, and used for the polarographic measurement of narceine. Two drops of gelatine solution are added, after which nitrogen is passed through the solution for 5 minutes. Measurements[5] are taken from — 0.8 volts at 20° C (E = 1/5); evaluation is performed in the usual manner, the narceine values being read off a calibration curve.

Figure 10



Graduation curves

1 papaverine (312 mμ; 1-cm vessel) 3 narcotine (312 mμ; 1-cm vessel)
2 morphine (470 mμ; 2-cm vessel) 4 narcotoline (410 mμ; 1-cm vessel)

[4] On addition of the aqueous ammonia or after heating, the solution usually becomes somewhat cloudy; filtration through a small circular filter is therefore sometimes necessary.

III. Calculation of Results

The amounts of alkaloid corresponding to the extinctions observed can be either read off calibration curves or calculated from the following ratios, obtained from the calibration factors given by the curves in figs. 10 and 11 :

$$\text{Morphine } \% = \frac{E_{1\ cm} \cdot 3.54}{a}$$

$$\text{Codeine } \% = \frac{E_{1\ cm} \cdot 0.317}{a}$$

$$\text{Thebaine } \% \frac{E_{1\ cm} \cdot 0.069}{a}$$

$$\text{Narcotine } \% = \frac{E_{1\ cm} \cdot 0.175}{a}$$

$$\text{Papaverine } \% = \frac{E_{1\ cm} \cdot 0.0695}{a}$$

$$\text{Narcotoline } \% = \frac{E_{1\ cm} \cdot 0.41}{a}$$

E_1 cm = extinction observed (calculated for a 1-cm vessel)
a = weighed portion in g.

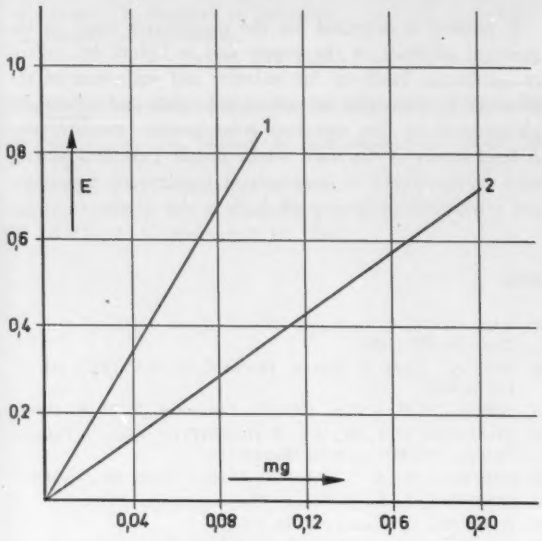
IV. Determination of Morphine in Isolated Poppy Capsules or Parts of Same

1. *First Method (photometric)*

0.1 to 0.2 g of poppy-capsule powder dried at 75°C. are heated in a centrifuge glass with 5 ml of N/10 acetic acid for ten minutes over a boiling water-bath, the whole being shaken from time to time. After cooling in cold water, 0.5 g of lead acetate is added, the centrifuge glass closed with a rubber stopper, and shaken hard for one minute. 0.5 g of dried sodium sulphate is added, the mixture again shaken hard, water added up to the level of about 10 ml, and the whole centrifuged for about three minutes at 3,000 to 5,000 revolutions per minute. The solution obtained in this way, which should be only slightly cloudy, is transferred to a separating funnel, while the residue is stirred and centrifuged twice more with 2.5 ml of N/10 acetic acid. The pH value of the combined solutions — which should be approximately 5.5 to 6.0 — is next determined with a suitable indicator paper.

[5] In these studies, a micropolarograph, Heyrovsky system, with photographic registration of the voltage curve, was used.

Figure 11



Graduation curves

1 thebaine (570 mμ; 1-cm vessel)
2 codeine (500 mμ; 2-cm vessel)

Where necessary, a small amount of sodium acetate may be added. The extract is next shaken twice, each time for two minutes, with 20 ml of chloroform, the chloroform solution is separated, and the solution to be analysed is mixed with 2 ml of 5% soda-lye and 0.3 g of ammonium chloride. The solution (pH approximately = 9) is extracted with 2 × 20 and 1 × 10 ml of chloroform-isopropanol (3 + 1 parts by vol.), each time for two minutes. After distillation of the solvents over a water-bath, the residue is absorbed with 5 ml of chloroform. From this, the morphine is drawn off with 10 ml of N/10 hydrochloric acid. After separation of the layers, the main portion of the hydrochloric solution is carefully poured off through a filter. 5 ml of the (clear, colourless) solution are placed in a test-tube fitted with a glass stopper, mixed with 2 ml of a 1% sodium-nitrite solution, and immediately shaken hard for 15 seconds. Exactly 15 minutes after addition of the nitrite, the sediment is mixed with 3 ml of 10% aqueous ammonia, and, after careful rotation, with 2.5 ml of water. After 5 more minutes, the extinction is measured against a standard solution consisting of 5 ml of N/10 hydrochloric acid and the above-mentioned amounts of nitrite solution, aqueous ammonia, and water (470 mμ, 2-cm vessels). The amount of morphine corresponding to the extinction observed may be read off a calibration curve. The morphine content of the capsules may be calculated from the formula

$$\text{Morphine } \% = \frac{a \cdot 2 \cdot 100}{b}, \text{ where}$$

a = the amount of morphine read off the calibration curve;

b = the measured portion.

2. *Second Method (photometric)*

0.2 g of finely powdered poppy capsules dried at 75°C are powerfully crushed in a mortar with 0.5 to 1 ml of water (in the case of coarser powder, 0.5 g of quartz sand should be added), and 15 minutes later partially ground fine with approximately 5.0 g aluminium oxide "acid". This gives a dry powder, which must be free from any coarse capsule parts. The powder is placed in a glass tube approximately 1.5 cm in diameter and 15 cm in length, the narrow end of which is closed with cotton wool and covered with approximately 3 g of aluminium oxide "acid". This is next washed out with water, which is fed drop by drop from a separating funnel placed on the tube in such a way that, at a speed of 20 drops per minute (regulated by means of a water-jet pump), a layer of water 1 cm deep constantly covers the aluminium oxide.[6] Some 20 ml of the extract are absorbed, rinsed with 2 ml of water, and transferred to a separating funnel. 1 ml of aqueous ammonia (DAB 6) and 0.2 g of ammonium chloride are added, after which extraction is performed with 2 × 20 ml and 1 × 10 ml of chloroform-isopropanol (3 + 1 parts by volume), for two minutes in each case. The combined solutions are heated over the water-bath while the solvent is distilled. The residue is absorbed with 5 ml of chloroform, after which extraction is performed with 15 ml of N/10 hydrochloric acid. 5 ml of the hydrochloric solution are then treated as explained under 1 above, for purposes of photometric measurement. A further 5 ml of this solution are mixed with 4.5 ml of water and 3 ml of aqueous ammonia and heated for 5 minutes in a boiling water-bath. This solution here serves both as a standard solution and also to compensate the narcotoline contained in the solution. The amount of morphine is calculated from the formula

$$\text{Morphine } \% = \frac{a \cdot 3 \cdot 100}{b}, \text{ where}$$

a = the amount of morphine read off the calibration curve;

b = the measured portion.

3. *Third Method (polarographic)*

0.3 g of finely powdered poppy capsules dried at 75°C are prepared as under IV, 2, above, and extracted, the amount of aluminium oxide being increased in this case to 7-8 g. Approximately 20 ml of the extract are absorbed and, after rinsing with 3.5 ml of hydrochloric acid (DAB 6), placed in a measuring flask of 25-ml capacity. Water is then added up to the mark. To 5 ml of this extract (= 0.06 g poppy-capsule powder), 2 ml of N/1 sodium-nitrite solution are added and, exactly 5 minutes later, 3 ml of 20% potash-lye and 7 drops of gelatine solution. Prior to polarographic measurement, nitrogen is passed through the solution for 5 minutes. During polarographic measurement, bottom mercury is used as the supply electrode from – 0.2 volts at 20° C. By using an electrolysis vessel as the polarographic cell, it is possible to perform analysis in a vacuum. The polarograms are calculated

[6] Apparatus 8,II has proved successful for this extraction process.

by extending the lines of the fundamental current and the limit current to right and left respectively. In addition, an auxiliary line is drawn as a tangent through the centre of the rising part of the curve, and the difference in height between the points of intersection of these tangents is measured against the extended lines of the fundamental and limit currents. Finally, the morphine values are read off a calibration curve. The entire process — including waiting time, but excluding photographic work — takes about 45 minutes.

Summary

A method is described for the quantitative assay of the principal alkaloids in the poppy and in opium by micro-measurement, based on the isolation and separation of the alkaloids by extraction at various pH-values and subsequent photometric or (for narceine) polarographic measurement. A brief survey of the main works already published dealing with the occurrence (= geographical distribution), formation, and content of the poppy alkaloids is also given.

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Determination of morphine in poppy capsules

By Giuseppe Pruner
Biological Laboratory, Istituto Superiore di Sanita, Rome

The method here described is based on the same principle as that described in an earlier note (1) on the determination of the morphine in opium. The results obtained were compared with those given by Wüest & Frey's weighing method (2) performed on the same poppy capsule samples by chemists of the Hoffmann - La Roche Company.

Table 1 summarizes Wüest & Frey's method, which is very time-consuming and requires a large quantity of organic solvents, and the much simpler and quicker cerimetric method. As to accuracy, the results obtained by the cerimetric method compared with the results obtained by the La Roche chemists can be considered satisfactory, as will be apparent from table 2. Although the first sample, No. 58, gave a negative result in Wüest & Frey's analysis, the presence of morphine was detected by the cerimetric method and confirmed by the industrial alkaloid yield.

The dry capsules of *Papaver somniferum* and of other varieties commercially known as poppy straw (Mohnstroh) are the waste parts — useful only as fuel — of the dry plant after its seeds have been removed for the extraction of an edible oil.

An industrial method for extracting morphine directly from the poppy plant was first evolved by the Hungarian scientist Janos von Kabay in 1933, and was developed in Poland, Germany, Russia and other European countries.

New and cheaper sources of edible oils have now reduced poppy cultivation in Europe to such an extent that the industries using this raw material are in difficulties.

The peasants of the Upper Etsch in Italy have grown poppies from time immemorial just for their seeds, which, when ground and sugared, are used in traditional confectionery such as, for instance, " Mohnstrudel ".

TABLE 1

Wüest & Frey's weighing method		Cerimetric method	
Pulverized poppy capsules, 200 g		*Pulverized poppy capsules, 5 g*	
1. Hot extraction with acid alcohol	1,000 cc	Extraction with H_2O	170 cc
Washing with alcohol	500 cc	Purification phases of the aqueous layer :	
2. Hot extraction with acid alcohol	1,000 cc	1. Filtration on acid aluminium oxide	20 g
Washing with alcohol	250 cc	2. Concentration to 20 cc and treatment with $Ca(OH)_2$	
Concentration in vacuo to 120 cc; treatment with CaO, H_2O q.s. for 400 cc; treatment with Na_2CO_3, HCl and Na_2CO_3		3. Purification with lead acetate	
3. Extraction with butylbenzene alcohol	750 cc	4. Treatment with sulphuric ether	20 cc
4. Extraction with 30 cc of 2% H_2SO_4. Washing with 30 cc sulphuric ether		Dilution with buffer solution having a pH factor of 10, addition of potassium ferricyanide solution, and titration with N/200 $Ce(SO_4)_2$, with ferroin as indicator	
Precipitation with Na_2CO_3. Washing, drying and weighing of the morphine			

Dry poppy capsules collected at 1,500 m in the Dolomites last summer were found to have a morphine content of 1.29‰, a very low value compared with the Hoffmann-La Roche samples.

Opium production in Europe has been the subject of experiments in a number of countries, and a good morphine content was obtained; but efforts to start up large-scale production failed because of high labour costs, since thousands of capsules (about 40,000 per kg of opium) have to be cut, and the opium collected afterwards.

The note (1) previously mentioned described the difficulties found in early efforts to apply the same method as is used to determine morphine in opium to poppy capsules, for they have a low morphine content of (on the average) 1‰ to 6‰, and contain many other substances which have to be eliminated. Apparently, these difficulties can be overcome by providing an acid Al_2O_3 filtration of the aqueous extract of the capsules.

The same note also mentions narcotoline, which was thought to be present only in poppy capsules, but was recently found by Pfeifer (3) to be a constituent of opium too.

Porphyroxine meconidine, on the other hand, which is present in opium in amounts depending upon the source country, is apparently not present in poppy capsules, as was found in an origin study based on the determination of the alkaloid pattern (4).

Experimental Part

Reagents and Solutions used

1. N/200 cerium sulphate solution, $Ce(SO_4)_2.4H_2O$: About 1.10 g of salt are dissolved, in a 500 cc measuring flask, in about 250 cc of water to which 14 cc of concentrated H_2SO_4 have been added. After complete solution the mixture is made up to the mark.
2. N/200 solution of anhydrous $K_4Fe(CN)_6$: This is used to titrate the cerium sulphate solution, and is prepared freshly for each use, 0.3683 g of salt being carefully weighed out and mixed in 200 cc of water.

   Titration is performed as follows : 8 cc of the solution are acidified with 2 cc of HCl (solution 6) and are titrated, the N/200 $Ce(SO_4)_2$ solution flowing from a 10-cc semi-microburette in the presence of three drops of ferroin (solution 5), which changes colour very markedly from reddish orange to greenish yellow.
3. 1% solution of $K_3Fe(CN)_6$.
4. Buffer solution :

Bisodium phosphate dihydrate	4.138 g
Anhydrous monopotassium phosphate	1.415 g
Anhydrous sodium carbonate	4.285 g
Distilled water q.s.	1,000 cc

5. M/40 solution of indicator (Ferroin). This is prepared by mixing 0.406 g of o-phenanthroline hydrochloride and 0.179 g of crystalline ferrous sulphate in 25 cc of distilled water. 1 cc is taken from this mother liquid and made up with water to 20 cc, the diluted solution being kept in a dropping bottle.
6. Hydrochloric acid (50 parts by volume of concentrated HCl and 50 parts of water).
7. 10% neutral lead acetate solution.
8. 3.75% anhydrous sodium sulphate solution.
9. Pure sulphuric ether free from peroxides and aldehydes.
10. Pulverized hydrated calcium.
11. Acid aluminium oxide for chromatography (C. Erba).

Description of the Method

5 g of poppy capsules in powder form are macerated for 15 minutes in a mortar with 50 cc of water; 5 g of acid alumina are added and mixing continued for 2 more minutes, when the contents of the mortar are poured into a centrifuge tube. After centrifuging at 2,000 rpm for 2 minutes, the liquid, together with the floating wood particles, is poured on to an 11 G 3 porous Jena glass filter containing 20 g of acid Al_2O_3 and connected to a vacuum flask. The liquid should filter slowly through the layer of Al_2O_3. Using a pestle-like glass rod, the residue in the tube is mixed with 40 cc of water added a little at a time. Centrifuging and filtering are carried out as above. This treatment is repeated twice. The contents of the vacuum flask are poured into a glass capsule and the volume of liquid is reduced to about 20 cc by boiling on a water-bath. The capsule contents are poured into a centrifuge tube, cooled, and centrifuged for 2 minutes. The liquid is then transferred through a filter paper into a 25-cc cylindrical vessel with a ground-glass stopper; the capsule and residue in the tube are washed with sufficient water to make up to the mark.

0.5 g of $Ca(OH)_2$ is added to the cylinder, mixing is carried out for 2 minutes and the cylinder contents are then poured into a second centrifuge tube, and centrifuged for 2 minutes. The liquid is then filtered through paper into a second cylindrical vessel, of 50-cc capacity, with a ground-glass stopper. After the 20 cc have been added, 4 cc of lead acetate solution are added, the cylinder closed and its contents mixed. 4 cc of sodium sulphate solution are introduced 5 minutes later to precipitate the excess of lead. 12 cc of buffer solution are poured into the cylinder to bring the volume to 40 cc and mixing is continued for a few minutes. Filtration is then carried out.

10 cc of filtrate are taken up in a pipette and placed in a wide tube of about 50 cc capacity with a ground-glass stopper; 10 cc of sulphuric ether are added and the mixture agitated for 2 minutes, preferably by means of an agitating mechanism. After a few minutes, the layer of ether is taken up by means of a piston pipette, a further 10 cc of ether are added, agitation is continued for 2 minutes, and the layer of ether removed. Should the liquid become cloudy, it can be cleared by slight suction at the water pump.

5 cc of this aqueous liquid are sampled, transferred to a 25-cc flask, diluted with 15 cc of buffer solution, and filtered through paper into a second flask. Using a 5-cc pipette, the filtrate is divided up into 3 25-cc beakers which are placed in a water-bath at 20°C if the room temperature is different. This helps to keep the temperature as well as the time of the reaction constant. Using a 10-cc semi-microburette, 1 cc of potassium ferricyanide solution is introduced rapidly into each beaker. The oxidation reaction is stopped after 10 minutes by introducing 2 cc of HCl (sol. 6) into each beaker. Three drops of ferroin are added to each vessel, and the ferrocyanide formed during the reaction is titrated with N/200 cerium sulphate solution, which is dropped in from a microburette until there is a clear change of colour from reddish-orange to greenish-yellow.

The rotating-field electric agitator can be used advantageously for titration, with 12-mm plastic-covered magnetic rods.

The morphine content of 100 g of poppy capsules tested can be deduced from the formula :

$$M = Ce \cdot \frac{8 \times 100}{2.454} = Ce \cdot 326$$

where Ce denotes the average cc of cerium sulphate used in 3 or more titrations (less 0.10 cc for the control) multiplied by 326; 2.454 represents cc of N/200 cerium sulphate solution, equivalent to 1 mg of morphine.

Check on the Method

Ten samples of poppy capsules from different sources were used for this purpose. They had been dried at 70°C and pulverized by Hoffmann-La Roche, and were despatched in

waxed dark glass bottles. The source of three samples was not shown, since they were mixtures of different batches.

Table 2 gives a comparison of the results obtained using the method described with the results obtained by Wüest & Frey's method, as used by the Hoffmann - La Roche chemists.

It will be noted that the cerimetric method gave lower values than Wüest & Frey's method in the case of the old samples dating from 1953, which would tend to confirm the observation made when analysing old opium samples that morphine oxidizes slowly in the course of time due, according to Abraham & Rae (5), to an oxidizing enzyme, opiase, leading to the formation of pseudomorphine $(C_{17}H_{18}O_3N)_2$ (6).

TABLE 2

No.	Sample	Method		Difference	
		Wüest & Frey	Cerimetric	Absolute	Percentage
		%	%		
58	Austria (1954)	0.00	2.07	—	—
	821 – 840 (26/9/56)	2.51	2.48	— 0.03	— 1.20
	801 – 820 (20/9/56)	2.54	2.48	— 0.06	— 2.36
32	Yugoslavia (1954)	2.81	2.85	+ 0.04	+ 1.40
	Mixture 1601-1602	3.39	3.45	+ 0.06	+ 1.80
26	Federal Republic of Germany (1955)	3.37	3.53	+ 0.16	+ 4.80
87	Federal Republic of Germany (1953)	3.70	3.62	— 0.08	— 2.20
16	Eastern Zone of Germany (1953)	4.18	3.95	— 0.23	— 5.50
33	Romania (1953)	4.64	4.26	— 0.38	— 8.20
36	Romania (1953)	4.45	4.22	— 0.23	— 5.20
		Average ...		± 0.14	± 3.63

The behaviour of this alkaloid in more conventional methods of opium analysis is being studied.

The reproducibility of the method is satisfactory, as will be apparent from the three analyses performed on sample 821-840 : 2.48; 2.52; 2.45.

Thanks are due to Dr. Saenger, General Director of the Deutsche Hoffmann - La Roche of Grenzach, and particularly to Dr. W. Wasmer for supplying me with the ten samples for carrying out this investigation and with the respective analytical results.

Summary

A known cerimetric method for determining the morphine in opium was applied with suitable modifications to the determination of the morphine in poppy capsules.

The aqueous extract of the drug is filtered through acid alumina, concentrated and purified by consecutive treatment with lime, lead acetate and ethyl ether.

The results of the analyses of ten poppy-capsule samples from different sources are compared with the results obtained when Wüest & Frey's weighing method was applied to the same samples by the chemists of the Deutsche Hoffmann - La Roche.

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Committee of Experts on the United Nations Programme of Opium Research [1]

In accordance with several resolutions of the Economic and Social Council, widespread efforts on national and international levels have been in progress for several years to develop a reliable method for determining the probable origin of opium seized in the illicit traffic. On the proposal of the Commission on Narcotic Drugs, the Economic and Social Council decided[2] that a seminar of not more than nine experts should meet in 1958 for a period of two weeks to review and evaluate this research.

The Committee met from 20 to 31 January 1958. It was composed of the following experts (serving in their personal capacities accompanied by their advisers) :

Mr. Haruyo Asahina, Chief, Narcotic Section, National Hygienic Laboratory, Tokyo, Japan

Professor H. Baggesgaard-Rasmussen, Chairman of the Organic Chemistry Department, Royal Danish School of Pharmacy, Copenhagen, Denmark

Dr. Charles G. Farmilo, Chief, Organic Chemistry Section of the Food and Drug Laboratory, Department of National Health and Welfare, Ottawa, Canada

Dr. A. H. Homeyer, Mallinckrodt Chemical Works, St. Louis, Missouri, United States of America

Miss Zisan Karaata, Chief Chemist, Opium Section of the Soil Products Branch Office, Istanbul, Turkey

Mr. Palamarneri S. Krishnan, Chief Chemist of the Central Revenues Control Laboratory, Department of Revenue of the Ministry of Finance, Government of India

Mr. L. N. Macleod, Commonwealth Analyst, Commonwealth Laboratory, Department of Customs and Excise, Melbourne, Australia

Dr. Georges Panopoulos, Honorary Director-General of the National General Laboratory, Athens, Greece; and Professor of Criminological Chemistry at the Police Training Institute

Professor Vassili V. Zakusov, Director, Institute of Pharmacology and Chemical Therapy, USSR Academy of Medical Sciences, Moscow, USSR.

The main questions they studied were the following :

Authenticated opium samples

The physical and chemical procedures for establishing the geographical origin of opium

Methods of making origin determinations (draft Code of Reference for the use of national laboratories)

Future organization of the work.

The present article gives an account of the work of the Committee.

AUTHENTICATED OPIUM SAMPLES

A. Question of opium types

It was observed that opium types had not yet been established. The Committee decided that this was a basic question on which it would be necessary to reach a definite conclusion. For this purpose it was felt to be necessary to define the term " opium type "; to delineate the various types; to indicate the geographical areas where each was produced, and to make the necessary arrangements for this work to be carried out. The following definition was adopted :

" An opium type is one whose physical and chemical characteristics lie within defined limits."

As regards this draft definition, the Committee noted that the variations in characteristics from year to year may increase or decrease the number of types within a particular country.

The character of each opium type should be elucidated by measurement of its chemical and physical properties, and the limits should be defined as closely as possible, taking into account all the available information.

Variations in moisture should not constitute a difference in type, and the various constituents should be calculated on anhydrous opium or on opium having a constant moisture content.

The need to establish the various opium types by defining the ranges for each of their characteristics was recognized. This would constitute an important step forward in the scheme for establishing opium origin.

It was noted that more authenticated samples were available from India than from any other opium producing country, yet only a tentative classification could be made for Indian types. As regards the other producing countries — from which fewer samples were available — difficulties were naturally much greater. The scientists stated that their inter-

[1] See also on that subject the three following articles in the *Bulletin on Narcotics* : " Determining the origins of opium " (Vol. I, No. 1); " The story of ' porphyroxine-meconidine ' " (Vol. IV, No. 1); " Progress in determining the origin of illicit opium " (Vol. V, No. 1).

[2] Resolution 626 H (XXII), *Twenty-second Session of the Economic and Social Council, 2 August 1956.*

national collaboration on opium could not progress to the final stage of delineating opium types until all the countries had supplied authenticated samples.

B. Supply of Opium Samples

The Committee considered as authenticated samples only those that had been "furnished and authenticated by the government of the country of origin as opium produced in that country".

A review was made of the countries and territories throughout the world where there was a question of licit or illicit production to determine how fully they were represented among the samples used for the research programme.[3]

(1) *Countries with Licit Production*

Afghanistan [4]

In 1956, licit production amounted to 12.2 tons. Three authenticated samples of Afghan opium were available.

Bulgaria

Bulgarian licit production varied between 6.7 and 1.1 tons during the period 1952-1956. No authenticated samples of Bulgarian opium were available.

Greece

Licit production, which was discontinued in 1953, would perhaps recommence in 1958. An authenticated sample of Greek opium was available.

India

India was one of the principal producers of licit opium. During the past five years, production varied between 347.7 tons in 1956 and 629 tons in 1953. One hundred and sixty-seven authenticated samples of Indian opium were available.

Iran

Beginning in 1956, licit opium production had been prohibited, and further intensive measures were taken to suppress illicit cultivation. Thirty-two authenticated samples of Iranian opium were available.

Japan

Opium production had been begun in 1954, and in 1955 consisted of 2.3 tons, and in 1956 of 2.0 tons. Fourteen authenticated samples of Japanese opium were available.

Pakistan

Licit production in recent years had varied between 4.6 tons in 1954 and 165 kg in 1952. Six authenticated samples of Pakistani opium had been received.

Turkey

Turkey was one of the principal producers of licit opium. Turkish production had varied recently between 466 tons in 1952 and 70 tons in 1954; in 1956, 277 tons were produced. Thirty-four authenticated samples of Turkish opium comprising 110 separate lumps had been made available, and additional samples were expected in the near future.

Union of Soviet Socialist Republics

The Union of Soviet Socialist Republics was the third largest producer of licit opium; production took place in the Kazakh and Kirghiz Soviet Socialist Republics, and during the past five years had varied between 92 tons in 1953 and 108 tons in 1955. No authenticated samples of Soviet opium were available.

Yugoslavia

Licit production of opium in Yugoslavia had varied between 12 tons (1952, 1954) and 28 tons (1956). An authenticated sample of Yugoslav opium consisting of a number of lumps which might or might not come from different producing areas had been received.

(2) *Countries with Illicit Production, having furnished Authenticated Samples*

Burma

There was illicit production in certain regions. Only one authenticated sample was available to the United Nations Opium Distribution Centre.

Korea, Republic of

The Government had reported some illicit production in 1956. One authenticated sample of Korean opium was available.

Laos

The Government had reported some illicit production. Five authenticated samples of Laotian opium had been made available in 1950.

Mexico

There was illicit production of opium in the western mountain districts; one authenticated sample of Mexican opium was available.

Nepal

One authenticated sample of Nepalese opium had been received, but no information was available on opium production, licit or illicit, in that country.

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[3] The following data are based on information circulated in United Nations documents — e.g., annual reports of governments, reports of the Permanent Central Opium Board — and on information provided by the participants or by the previous committee of experts.

[4] In the meantime, a note has been received from the Government of Afghanistan transmitting officially to the Secretariat of the United Nations the text of a law of 24 November 1957 concerning the prohibition of cultivation, trade, purchase, sale, import, export and use of opium in Afghanistan; the cultivation of the opium poppy and the export of opium will henceforward be prohibited (doc. E/NL.1958/13).

Viet-Nam

The Government reported in 1956 that illicit opium production in Viet-Nam had been completely suppressed. One authenticated sample of Viet-Namese opium consisting of a number of small lumps which came from the same or different localities was available.

(3) *Countries which have not furnished Authenticated Samples*

Ecuador

Production was illicit, and no authenticated samples of Ecuadorian opium were available.

Lebanon

The Government stated in 1956 that cultivation of opium poppy was not known in Lebanon. If there was production of illicit opium at other times, no authenticated samples were available.

Thailand

There was some illicit production. No authenticated sample of Thai opium had been received.

Tunisia

The Government have reported some illicit cultivation of the opium poppy, but authenticated samples of Tunisian opium, if any has been produced, have not been received.

United Arab Republic

Production was illicit, and no authenticated samples of opium were available.

* * *

No recent information was available for the following countries : China and Peru.

In some instances, supporting information serving to identify the exact geographical origin of many of the authenticated samples was lacking, and also the dates and circumstances of harvesting and storage were unavailable.

The Committee accordingly decided to recommend to the Commission on Narcotic Drugs that the governments of the above-mentioned countries should be urged to furnish many more authenticated samples, and that each sample, in so far as possible, should be accompanied by the following detailed information :

(i) Year of production;
(ii) Precise locality of production;
(iii) Details of harvesting — e.g., first or second lancing;
(iv) Weight of the sample;
(v) Whether it is opium from one cultivator or several neighbouring cultivators;
(vi) Local name of the variety of poppy;
(vii) Other relevant data.

It was recognized that the foregoing details might not be available for authenticated samples of illicitly produced opium that might be confiscated and made available, but it was hoped that as much information as possible regarding those samples would also be furnished.

The Committee also decided to recommend that governments should be asked to furnish samples from opium seizures even in cases where they had not been able to authenticate them as having been produced within their territories. Such samples might indicate the existence of new opium types, and could be compared with one another and with the existing authenticated samples. Such information would also be of use to narcotics control authorities.

For establishing types, it was considered ideal that samples should be collected where possible from each of the producing localities for a period of five years; it was estimated that the total number of samples collected over this period would be approximately 1,000.

C. Distribution of Authenticated Opium Samples

The Committee recognized that the requirements of laboratories participating in the research programme had to be distinguished from those which were solely engaged in making origin determinations on the basis of recommended methods. All active participants in the research programme should, so far as possible, have all the samples they requested made available to them.

As regards the " national " laboratories making origin determinations, it would be sufficient if they possessed a representative collection of samples including the principal opium types, although this collection would probably constitute only a relatively small percentage of the total number of authenticated samples that might be available.

THE PHYSICAL AND CHEMICAL PROCEDURES FOR ESTABLISHING THE GEOGRAPHICAL ORIGIN OF OPIUM

The Committee reconsidered each of the methods which had been used or proposed for use in making origin determinations and evaluated each method in relation to its accuracy, reproducibility and usefulness for this purpose as well as the time, equipment and material involved in carrying out each method. These methods included both those in current use and others still under investigation by the scientists engaged in the United Nations programme.

The word " method " was defined as follows : " A method is an indication in outline of the characteristic or characteristics to be determined."

The following methods were reviewed and evaluated for purposes of establishing a scheme for opium origin determinations.

A. Macroscopic and Microscopic Examination

(1) *Macroscopic Examination*

The characteristics of opium, established by ordinary careful observation, were considered to be of value as a preliminary test.

The following points should be considered :

Kind of sample : Raw, extract, marc, etc.;
Adulteration : Hairs, bark, etc.;
Colour : Light, medium, dark-brown, black, etc.;
Form : Lumps, powder, sticks, moulded cakes, etc.;
Texture : Dry, irregular or smooth fracture, oily, presence or absence of leaves or bits of straw and soil, etc.;
Covering : None, leaf, paper, etc.;
Odour : Normal, sour, of ammonia, etc.

(2) *Microscopic Examination*

The advantages of the microscopic examination were its rapidity, simplicity and use of little material and readily available equipment. The test had always to be used in conjunction with other methods of origin determination.

B. Methods for determining alkaloids and other Organic Components of opium — Individually and in Ratio

(1) *Unified Methods for Alkaloidal Determination*

At least three unified methods had been devised for the determination of morphine, codeine, thebaine, papaverine, narcotine, porphyroxine-meconidine and minor phenolic alkaloids. The Committee decided that for present purposes it would adopt the method as described in document ST/SOA/SER.K/34 entitled " A Unified Analysis of Opium for Alkaloids ".

The results so far available for the five major alkaloids were of considerable diagnostic value, and would become more important as additional data accumulated. Although the absolute values of the individual alkaloids were regarded as important, the relative quantities of alkaloids and the ratios of one alkaloid to another yielded more useful diagnostic information.

The Committee noted the improved separation of papaverine and narcotine by use of ammonium reineckate, and learned that this method would be reported on in due course.

(2) *Paper Chromatography and Paper Electrophoresis*

There have been advances during the last several years in the techniques of paper chromatography applied to opium; these methods have the advantage of being rapid, requiring little complicated apparatus and small amount of sample. The methods might be divided into two groups : (i) those which give qualitative results; (ii) those which give quantitative values.

(3) *Qualitative Paper Chromatography and Electrophoresis*

These methods enabled qualitative comparisons of several opium samples to be made at one time, and thereby gave useful information for the determination of type. The value of the simple paper chromatographic and electrophoretic procedures was that they provided the analyst with many points of comparison on an opium sample which were useful for the general purpose of opium origin determination.

The Committee noted the usefulness in opium analysis of the simple chromatographic and electrophoretic methods.[5]

(4) *Quantitative Paper Chromatography and Electrophoresis*

Recent contributions on quantitative paper chromatography and paper electrophoresis had been received. These publications dealt with the analysis of total extracts of opium and of solutions containing all the alkaloids isolated from opium, in combination with spectrophotometry and densitometric determinations of the alkaloids.

These methods were rapid, required small samples, and provided a visible quantitative record of the different constituents. In addition, the analyst could handle a large number of samples in a single operation.

The accuracy and reproducibility of those methods remained to be determined, but the Committee was of the view that they would be of considerable value in the determination of opium types and in opium research generally.

(5) *Direct Ultraviolet Absorption Spectrophotometry*

Direct ultraviolet absorption spectrophotometry of solutions of opium [6] was studied in considerable detail. The Committee noted the distinction between the use of direct ultraviolet absorption spectrophotometry and spectrophotometry in conjunction with chromatographic and other procedures.

The direct ultraviolet absorption spectrophotometry might be usefully applied to the discrimination of opium types, but required further study. The method had been shown to be simple and rapid, and required small amounts of sample. The reproducibility between laboratories could be determined by collaborative assay.

The relationship of the ratios of extinction values and their correlation with origin were considered.

(6) *Simple Colour Reactions*

A preliminary report [7] had been presented on a method which depended on developing colours with various chemical reagents under specified conditions and measuring the colour intensity numerically.

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[5] *A Study of Paper Chromatography*, by G. Panapoulos & A. Vassiliou, Greece, ST/SOA/SER.K/27; *Supplementary Study on the Method for the Determination of Origin of Opium based on Paper Chromatography*, by G. Panapoulos & A. Vassiliou, Greece, ST/SOA/SER.K/37; *A supplementary Study of Paper Chromatography for the Determination of the Origin of Opium*, by G. Panapoulos & A. Vassiliou, Greece, ST/SOA/SER.K/42; *Chromatographic Analysis of Opium, its Alkaloids and Synthetic Narcotics for Identification Purposes*, by C. G. Farmilo, K. Genest, E. G. Clair, G. Nadeau, G. Sobolewski & Miss L. Fiset, Canada, ST/SOA/SER.K/58; *Paper Electrophoresis of Opium and its Use in solving Opium Origin Identification problems*, by C. G. Farmilo & W. P. McKinley with J. C. Bartlet, Mrs. P. Oestreicher & Mrs. A. Almond, Canada, ST/SOA/SER.K/61.

[6] *The Determination of the Origin of Opium by Means of Ultraviolet Absorption Spectrophotometry*, by L. Grlić & J. Petricić, Yugoslavia, ST/SOA/SER.K/48; *Determination of the Origin of Opium by means of Ultraviolet Absorption Spectrophotometry, II*, by L. Grlić, Yugoslavia, ST/SOA/SER.K/54.

[7] *A Preliminary Report on the Use of Colour Reactions for determining Origin*, by United Nations Secretariat, ST/SOA/SER.K/65 and Corr. 1.

The Committee considered that the advantages of the method were that it was short and simple, and required no separation of the opium constituents. The method was useful and promising, and should be studied further to determine its reproducibility and reliability for purposes of opium determination.

(7) *Methods for determining Other Organic Components*

After discussing the determination of nitrogen in opium and in certain opium fractions by the Kjeldahl method [8] and the analysis of opium for meconic acid and other organic constituents such as lipids, amino acids, the Committee thought that except as regards meconic acid and nitrogen, there were insufficient data for the purpose of evaluating the relationship between opium origin and the concentration of the other organic constituents of opium referred to in this sub-section. The ratio of narcotine to meconic acid might be of value in determining the origin of opium, but further work on this aspect was necessary.

C. Methods for determining inorganic Components of Opium — Individually and in Ratio (the Ash Methods [9]

It was observed that not all laboratories were furnished with spectrographic equipment and trained personnel to use this equipment, but that most laboratories had flame photometers, and for that reason the determination of potassium, calcium and phosphate in the ash would be possible. Although the methods were fundamentally the same, a distinction was made on the basis of the equipment used in each method.

The procedures for the ash methods were considered under the following headings: Preparation of sample; Flame photometry of the major elements; Colorimetric determination of phosphate; Spectrography of the major and minor elements.

Preparation of Sample

It was observed that the ash sample might be prepared either by dry calcination or wet decomposition in either platinum or silica dishes. The Committee decided that for purposes of opium origin determination, the dry calcination procedure was probably satisfactory if the temperature of calcination did not exceed 450°C. It was pointed out that in certain cases there could be loss of copper or phosphate in dry ashing procedures. However, this loss had not been established in the case of opium.

The Committee felt that for purposes of the collaborative tests on the method, the dry ashing procedure should be followed.

Recorded literature [10] showed that there was no loss of phosphorus in the opium dry ashing procedure.

Flame Photometry of Major Elements

The flame photometric method was very useful for purposes of establishing the origin of opium, and its accuracy was comparable with standard chemical procedures. The question of interaction of potassium, sodium and calcium and its effect on the accuracy of the results was also considered. It was noted that the published data [11] had been corrected for such interactions. It was pointed out that it would be advisable for laboratories undertaking flame photometry to participate in the collaborative assays for purposes of comparison of data from individual laboratories. It was essential that this method should be used in conjunction with other methods when establishing the origin of opium.

The Committee noted that it had been found possible to correlate very closely the origin determination by the composition of the ash with the origin determination by alkaloidal composition and other chemical methods. However, opium whose ash was abnormal due to special phenomena such as adulteration, contamination or the effect of fertilizers might be found. If such unusual results were observed, allowance would have to be made for them.

No data were available regarding the effect of artificial fertilizers on the ash of opium. It was not known whether the use of artificial fertilizers would bring about any appreciable change in the composition of ash, but if such changes were reported in the case of authenticated samples of opium, due note would have to be taken thereof in using this method for the determination of the origin of opium.

Colorimetric Determination of Phosphate

The ratio of potassium plus calcium plus sodium divided by phosphate (expressed in equivalent percentages) and the ratio of potassium divided by phosphate (expressed similarly) were valuable criteria for the determination of the origin of opium.

Spectrography of Major and Minor Elements

The spectrographic method for the determination of the major and minor elements present in opium ash had been useful in providing additional information for purposes of establishing the origin of opium when used in conjunction with methods for the analysis of other opium constituents. The equipment required for the procedure was expensive and not available in many laboratories, and therefore the use of the spectrograph was not made obligatory in the tentative scheme for establishing country of origin.

[8] *A Study of the United Nations Samples of Opium*, by G. Panapoulos & A. Vassiliou, Greece, ST/SOA/SER.K/16.

[9] *The Determination of Countries of Origin of Opium Samples by means of the Composition of the Opium Ash*, by J. C. Bartlet & C. G. Farmilo, Canada, ST/SOA/SER.K/30; *Spectrographic Analysis of Opium Ash*, by United Nations Secretariat, ST/SOA/SER.K/63.

[10] " Studies on the Ash Constituents of Indian Opium " from *Memoirs of the Department of Agriculture in India, Chemical Series*, 8, 45, (1925-1927), by H. E. Annett & M. N. Bose.

[11] *The Determination of Countries of origin of Opium Samples by means of the Composition of the Opium Ash*, by J. C. Bartlet & C. G. Farmilo, Canada, ST/SOA/SER.K/30; *Tabulation of results of Physical and Chemical Analyses of Authenticated Opium Samples*, by United Nations Secretariat, ST/SOA/SER.K/41; *Further Alkaloidal and Opium Ash Composition data for Determination of Opium Origin*, by C. G. Farmilo, J. Bartlet, P. Oestreicher & A. Almond, Canada, ST/SOA/SER.K/47.

METHODS OF MAKING ORIGIN DETERMINATIONS

The Committee agreed that it was now possible to make determinations of a limited kind of the origin of opium by interpretation of available data. Since there was no single method which would itself satisfactorily establish the origin of opium, it was necessary to use the results of several methods or combinations of methods to establish origin. The Committee therefore decided that it would try to develop a tentative scheme for establishing the country of origin of opium (draft code of reference for the use of participating laboratories) :

The Committee gave consideration to all of the methods that had been published to date for this purpose.

Tentative Scheme for establishing Country of Origin of Opium

A. Initial Procedure

Documentary information : Recording of the documentary information concerning the sample on punch card or report form.

(1) *Opium sample numbers*

The three cells of the punch card set aside for numbering should show :

(*a*) In the case of samples of seized opium, the number allotted by the country which made the seizure, the United Nations Laboratory number, and finally the number allotted by the laboratory which makes the analysis;

(*b*) In the case of authenticated samples, the first number should be that allotted by the country in which the opium was produced. The other two numbers would be as in (*a*) above.

If necessary, other numbers allotted to the sample should be quoted — e.g., the case number.

The data should be entered in indelible ink.

(2) *Dates*

(i) Sample received in laboratory;

(ii) Sample origin determined;

(iii) Sample origin reported.

(3) *Country and locality of origin for authenticated samples and, in case of contraband samples, place of seizure*

(4) *Informative data, sample history and description*

(i) For each seizure sample, an abbreviated case history;

(ii) For each authenticated sample, details of crop year, lancing, poppy variety;

(iii) For evidence of origin, separate the authenticated samples for which full information can be given and state, if possible, the circumstantial evidence of origin for seizures.

B. Preliminary Analytic Steps

Macroscopic examination (see above, p. 39). Preparation of sample for analysis : breaking up and drying of the sample at a temperature between 60°C and 70°C until completely dry so that it can be pulverised in a mortar.

C. Methods

Microscopic appearance.

Simple chromatography on paper.

Unified analysis and/or short methods, *British Pharmacopoeia* method, Anneler method, ammonium reineckate method.

Electrophoresis, a method recently described,[12] which might prove useful in determination of origin when studied further. It seems to offer the possibility of comparing a number of samples at the same time.

Simple colour reactions (see above, p. 40).

Direct ultraviolet absorption spectrophotometry (see above p. 40).

Kjeldahl nitrogen method (see above, p. 41).

Ash methods (see above, p. 41).

D. Record of results of analysis

It was essential for the establishment of opium types and origin determination on seized opiums to adopt the same procedure for recording analytic results in all laboratories.

E. Interpretation of Data : Alternative Systems for classifying Opium Types

One of the essentials necessary for the determination of the origin of unknown samples that may arise in contraband traffic was to establish types of authenticated samples from different countries. For this purpose it was essential that the scientists should have at their disposal as much qualitative and quantitative data as possible of each authenticated type. All the data that were collected must be recorded so that they could be readily employed for assigning any type that they may come across in the course of analysis. Four of the possible methods of arranging the data and of arriving at a conclusion about the correct type are listed and described briefly below :

(*a*) System of description;

(*b*) System of classification or alphabetical coding;

(*c*) System of alternatives;

(*d*) System of correspondence.

In the following paragraphs, a brief description of each system is given.

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[12] *Paper Electrophoresis of Opium and its use in solving Opium Origin Identification Problems*, by C. G. Farmilo & W. P. McKinley with J. C. Bartlet, P. Oestreicher & A. Almond, Canada, ST/SOA/SER.K/61.

(a) *The System of Description*

This system consists of writing descriptions of the types of opium to be provisionally recognized on the basis of existing data, emphasizing their differences and distinctive points. Each new authenticated sample is examined to see whether its outstanding characteristics conform to one of the provisional types, or whether they are significantly different. This system is thus analogous to the botanical classification of plants. It appears to be relatively simple, although it will be necessary to provide a uniform nomenclature for description. This would presumably be done on a collaborative basis.

(b) *The System of Classification or Alphabetical Coding*

In this case, the opium samples that have been analysed are provisionally classified by means of graphs which show the relationship between two or more sets of characteristics. The total area of the graph is subdivided into convenient sections by means of lines between the points and each of these sections is given a code number or a letter. The data for newly authenticated samples are then translated into the appropriate code on the basis of the analytical value, in order to see whether they have the same complete code designation. For example, the letters A J S might designate particular areas of the Calcium-Ratio 1 Chart.[13] One difficulty involved in the use of this method is the fact that some samples may lie close to the subdivision lines on the charts. It also sometimes operates to give a newly authenticated sample, a previously unused code designation, which may or may not mean that it is a new type. The method of coding may have to be developed further, when more samples have been examined.

(c) *The System of Alternatives*

This system consists of trying to find out how the results obtained on a new sample fit into the various groups of data within the existing groups, and to see that each sample fits as many points as possible for a given type.

(d) *The System of Correspondence or Matching*

This system of establishing identity of an unknown is the best known method, and is used universally in the field of organic analysis. It consists of matching the properties and characteristics of an unknown with those of the standard or reference. The punch-card method is based on this method of identification, and carries out the procedure in a mechanical way.[14] When the ranges have been determined, the procedure is automatic. Each newly authenticated sample is then "matched" in accordance with the prescribed rules of sorting. Some newly authenticated samples will have no corresponding known matching samples, in which case they would have to be established as belonging to new types.

[13] *The Determination of Countries of Origin of Opium Samples by means of the Composition of the Opium Ash*, by J. C. Bartlet & C. G. Farmilo, Canada, ST/SOA/SER.K/30, fig. 10, p. 34.

[14] *A Punched-card Method for Opium Origin Determinations*, by J. C. Bartlet & C. G. Farmilo with G. Taker, Canada, ST/SOA/SER.K/56.

The Committee decided that the essential methods would be listed, and that these methods would be considered as mandatory for any laboratory which desired to carry out opium origin determinations and which possessed the necessary equipment. The essential methods were divided into two groups on the basis of the availability of equipment for carrying them out.

The first group, consisting of methods which could be carried out by all laboratories, comprised :

(i) A punch-card method for facilitating a record of documentary information and analytical data;
(ii) Macroscopic examination;
(iii) Microscopic appearance;
(iv) Unified analysis of major opium alkaloids, including morphine, codeine, thebaine, porphyroxine-meconidine, papaverine and narcotine.

The second group of methods, which at present could be carried out only by laboratories having the necessary facilities, comprised :

(i) Spectrography;
(ii) Direct ultraviolet absorption spectrophotometry;
(iii) Electrophoresis;
(iv) Quantitative paper chromatography.

In view of the fact that these methods are considered to be of equal importance to those included in the first group, the Committee decided to recommend strongly that the various governments concerned should be invited to acquire the equipment and to train or acquire the personnel needed to carry them out.

The Committee emphasized the necessity for developing simple methods which should be of use to all laboratories, and considered that the method of simple colour reactions[15] showed considerable promise because of its inherent simplicity and should be tested on all authenticated samples as a routine procedure. It was noted by the Committee that a punch-card system had been used for purposes of relating simple colour reactions to origin of opium.

The Kjeldahl method for the determination of nitrogen in opium and fractions of opium was also considered of use in origin determinations.

It was emphasized that the four methods included in the first group should be used in combination with any of the other methods which were available to a given laboratory. It was contemplated that many of these methods would be evaluated by means of collaborative studies, and the results of such studies might make a re-evaluation of the methods necessary.

The Committee emphasized that it might be possible to replace some of the longer and more involved methods with simpler ones, and noted that important research was going on with this object in view.

[15] *A Preliminary Report on the Use of Colour Reactions for determining Origin*, by United Nations Secretariat, ST/SOA/SER.K/65 and Corr. 1.

FUTURE ORGANIZATION OF THE WORK

The Committee felt that the results of past research had led to a point where limited application — in the sense that the conclusions had to be considered provisional in many cases — was possible, and observed that the United Nations Narcotics Laboratory was well equipped with facilities for carrying out origin determinations (practical application) and research work. In the organization of the work programme, it was suggested that some equitable division of efforts be made between practical application of known methods and research for the improvement and extension of methods and the discovery of new ones.

As regards the work of the national laboratories, the Committee wished to encourage each of them to collaborate in the undertakings suggested in this report — e.g., collaborative studies — and also to extend their work in those directions in which they were particularly interested and skilled. The national laboratories were also invited to report their results in papers which should give as much detail as possible of the scientific procedures employed, and should conform with the mode of presentation of contributions to publications within the country from which the paper was submitted.

The Committee distinguished between three major fields of work in each laboratory, as follows :

A. Collaborative studies;
B. Practical application of methods to establish the origin of illicit samples of opium;
C. Research on methods.

A. Collaborative studies of Methods for Opium Origin Determination

To establish the precision and reproducibility of the unified analysis method, the spectrographic and flame photometric methods, the direct ultraviolet absorption spectrophotometric method, and simple colour reactions method, the Committee decided that the participating laboratories and the United Nations Narcotics Laboratory should undertake collaborative studies of these methods.

B. Practical Application

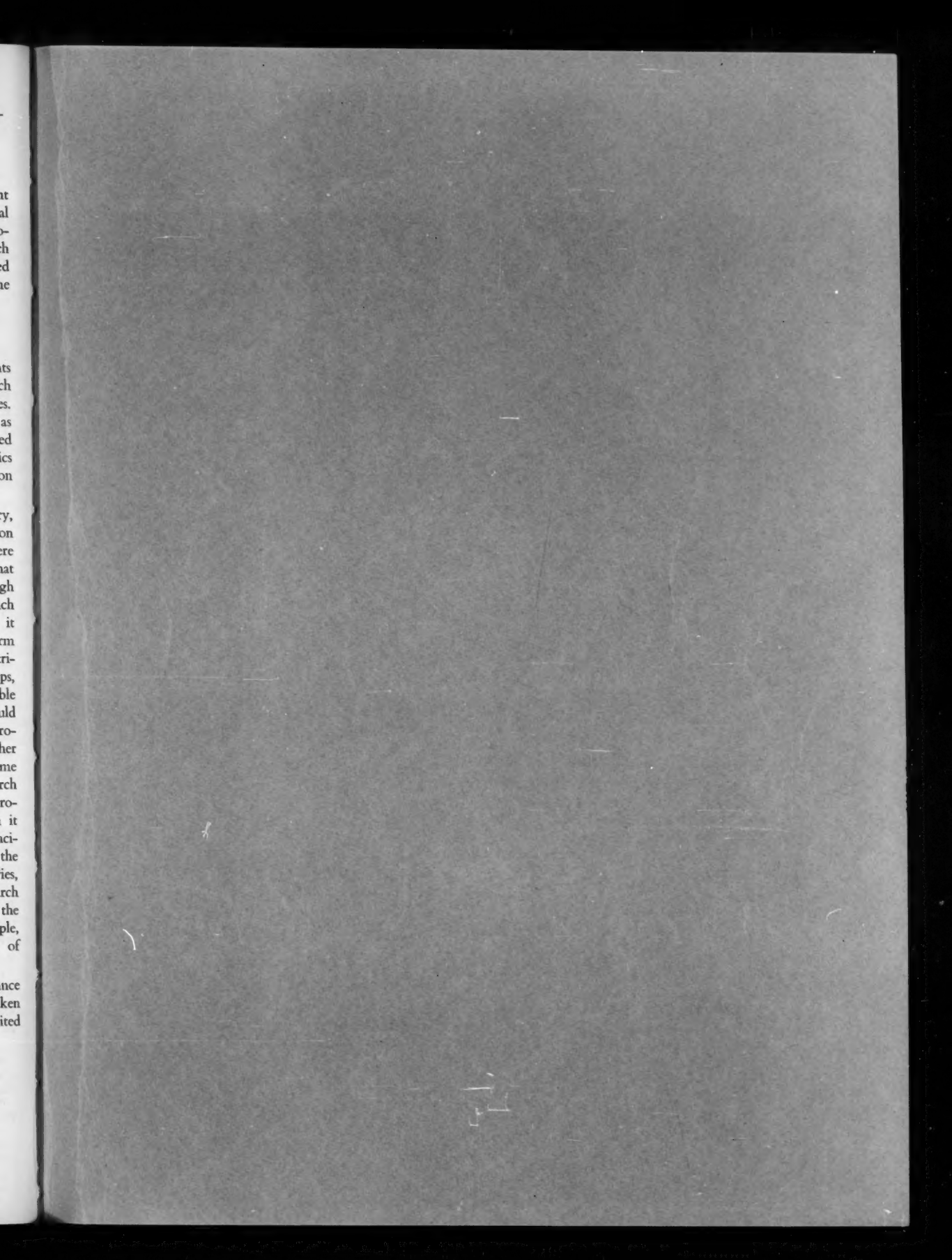
It was noted that practical application was being carried out in national laboratories, and that it would have to continue. It was also felt that the United Nations Laboratory should carry out origin determinations, but that these might generally be restricted to those samples on which there was a divergence of views and to those samples sent by governments which did not possess their own testing facilities. It was also pointed out in discussions that some national laboratories might wish to send samples of illicit opium either to other national laboratories for consultation or to the United Nations Laboratory as a reference case. Samples of seized opium which had been analysed by the national laboratories and the United Nations Laboratory should be retained for a very long time before they were destroyed or otherwise disposed of.

C. Research on Methods

The Committee found that it was difficult for participants in the programme to keep informed of the kind of research that was being carried out in each of the national laboratories. It defined, therefore, the several broad fields of work as follows : (i) research towards simplification of established methods; (ii) research on means of classifying characteristics with a view to establishing opium types; and (iii) preparation of a book of methods for opium origin determination.

As for the role of the United Nations Narcotics Laboratory, it was pointed out that it maintained an opium distribution centre, and distributed the scientific papers which were published in the ST/SOA/SER.K/... series. It was felt that the United Nations Laboratory should carry out enough analyses on seizures to keep in touch with the methods which were being developed in the total programme, and that it should endeavour to supply data in the best possible form for use of the national laboratories. In regard to the distribution of research problems among the participating groups, it was considered that the Secretariat could make a valuable contribution by issuing annual progress reports. This would enable the participants to evaluate their own research programme in relation to programmes being carried out in other countries. It should be possible for the Secretariat to give some advice in the establishment of priorities in respect to research problems. On request of participants in the research programme, the Secretariat could suggest problems which it thought were of importance. It was felt that in order to facilitate this it would be best for the Secretariat to examine the current publications in the ST/SOA/SER.K/... series, and to try to establish the specific fields in which research was being carried out. The Committee realized that the tasks of the national laboratories in most cases were multiple, and that opium research constituted one of a number of tasks which those laboratories had to carry out.

The question was also raised whether financial assistance could be provided in support of research work undertaken in participating laboratories for the benefit of the United Nations programme.



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